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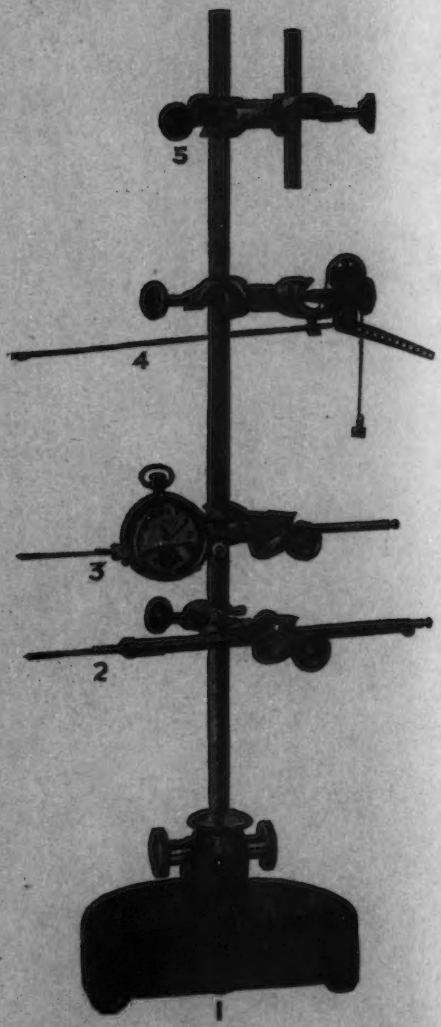

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JOSEPH BECKER

437 West 59th Street **New York City**

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VOL. 77 AUGUST 1, 1926 No. 3

LONG FLUCTUATIONS IN VOLUNTARY ACTIVITY OF THE ALBINO RAT[1]

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We have previously shown (Slonaker, 1924, 1925a, b) that the female albino rat exhibits marked fluctuations in voluntary daily activity which coincide with the oestrual rhythm and occur about every fourth day during sexual life. This was also described by Wang (1923) and by Durrant (1925). This rhythm was not found in sexually immature females nor in old females which had passed the menopause. Such a rhythm was also not demonstrated in males nor in ovariectomized females. Any conditions, such as pregnancy, pseudopregnancy, lactation, food, etc., which disturb the oestrual cycles likewise modify the character of this activity rhythm.

When the amount of daily activity over long periods of time is studied one notices long fluctuations, of weeks' or months' duration, which are wholly independent of the oestrual rhythm. We would expect that such fluctuations in average daily activity would occur in reproducing mothers owing to the effect of pregnancy and lactation reducing the voluntary activity to be followed by a great increase in activity after weaning the young. But when one finds similar long fluctuations in activity in all virgin rats studied and also in a group of males, one is led to conclude that these fluctuations are normal in the life history of the rat.

Brief mention of these facts was made in a former paper (Slonaker, 1907). Owing to a possibility that investigators studying the activity of the rat as modified by certain conditions may not take these normal fluctuations into consideration in reaching their conclusions, it is deemed wise to give the results of more extended observations.

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and The Committee for Research on Sex Problems of the National Research Council.

The following data were compiled from the activity records of three first-cousin bachelor rats and a first-cousin virgin female of the same age and of five sister virgin rats. The experiment on the males and single female (expt. 1) was carried on at an earlier date than that of the five virgin females (expt. 2). The diet also differed in the two experiments. The animals in experiment 1 were all fed the same food daily, which consisted of table scraps and cracked corn and water. The females (expt. 2) were given the same carefully prepared synthetic food throughout the experiment. Owing to the difference in time, in the location of the cages, and in the food a direct comparison between the two experiments cannot be made.

Each of these animals was placed in a separate revolving cage which was provided with an automatic revolution recorder. These cages were the homes of the rats during the remainder of their lives. During each of the experiments the environment was kept as nearly constant as possible throughout the life span. Differences in activity in the animals of each experiment must have been due to the inherent tendencies of the animals. Changes in climatic conditions, such as humidity, barometric or temperature fluctuations would presumably affect each animal of the experiment in a similar manner; a study of the data, however, excludes climatic changes as having any noticeable influence on these long fluctuations in the average daily activity.

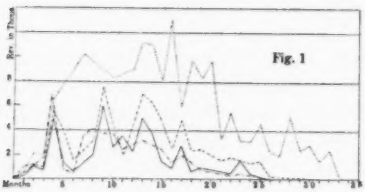

Fig. 1. Curves of activity of rats in experiment 1. Each curve represents the average of 30 days' activity. The dotted line is the female; the continuous line is male 1; the dash line, male 2; the dot-dash line, male 3.

Experiment 1. The three males and one female, which constituted this group, were placed in their respective cages at the age of twenty-eight days. They all showed an increase in activity during the first two months. With the exception of one male, no. 4, each showed a decreased activity during the third month. This was followed the fourth month by an almost uniform increase. This is shown in graphic form in figure 1. In constructing this figure the average run for thirty days was used to determine the length of the ordinates. By consulting this figure it is readily seen that after the fourth month the female greatly surpassed any of the males in activity during the remainder of her life. This is a common characteristic difference in the activity of the two sexes and has been previously described in detail (Slonaker, 1912). This figure also shows that each rat showed a number of marked increases in activity which were not synchronous. A tendency toward synchronism of high points was more common in the males than in the two sexes. But even in the males there

were enough exceptions to indicate individual variation. With the exception of the thirteenth month (possibly the fourth month also) when each rat showed increased activity there was no definite correspondence in the time of the fluctuations. This indicates in general that changes in environment have little if any effect in causing these fluctuations in activity. During her lifetime the female exhibited eleven, possibly twelve, fluctuations in activity and the males eight, eight and six each. The order of sequence is shown in table 1, x. Examination of this table shows that the time interval between peaks varied between two months and six months. The two month interval was the most common having occurred sixteen times, or 47 per cent. The per cent of times other inter-

TABLE 1

Showing the sequence of fluctuations of activity, x, in experiment 1

	MONTH																														NUMBER
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	
Female.....		x		x?			x					x				x		x		x		x			x			x		x		x	11–12?
Male 1.....		x		x					x		x		x				x		x				x										8
Male 2.....		x		x					x				x				x		x			x			x								8
Male 4.....				x				x		x			x				x						x										6

TABLE 2

Showing the sequence in activity, x, of each rat in experiment 2

	AGE IN DAYS																		NUMBER
	145	185	225	265	305	345	385	425	465	505	545	585	625	665	705	745	785	825	
A-1				x				x		x		x							4
A-2					x		x				x			x					4
A-3	x				x				x			x			x				5
A-4				x						x						x		x	4
A-5		x		x				x			x				x		x		6

vals occurred is as follows: three months, 20 per cent; four months, 17 per cent; five months and six months, a little less than 6 per cent each. Each animal showed wide variations in regard to the time interval between fluctuations. This table also shows a decided tendency toward a rhythm of approximately two months' duration in these long fluctuations in activity. The almost complete lack of synchronous arrangement strongly suggests that these fluctuations are inherent in the rat and are of normal occurrence.

Experiment 2. The five sister virgin rats constituting this group were placed in their respective cages at the age of 63 days. At this time none of these animals had reached pubescence. They were fed throughout the experiment on the following carefully prepared synthetic diet: ground

whole wheat, 3375; whole milk powder, 500; commercial casein, ground fine, 750; sodium chloride, 50; calcium carbonate, 75; ground alfalfa, 150; butter fat, 250. The dry ingredients were thoroughly mixed and the butter fat added and stirred in. The mass was then run through a grinding mill and again stirred. In this manner we felt sure that each small mass contained all the ingredients in the proportions given. The animals were fed daily greater amounts than they would eat. This enabled us to ascertain the daily intake of food for each rat.

The average voluntary activity, food consumption and weight of each of these rats at different ages are shown in figure 2, A, B, C, D and E respectively. In constructing the curves of activity the average daily run for the previous forty days was used in determining the length of the ordinate. This number was chosen because it is a multiple of the normal oestrual period and contains approximately the same number of oestrual cycles. It also gives a more uniform curve. A similar procedure was used in constructing the curve of food intake. The weight curve represents the weight of the animal at the age indicated. The sequence of fluctuations in activity is shown in table 2, x.

Examination of the curves of activity and the sequence of the fluctuations shows a complete lack of synchronism. There was a close approach to synchronism between the ages of 265 and 305 days, and suggested groupings at about the ages of 505 and 705 days. These three groupings, approximately 200 days apart, are only suggestive since each of these animals showed other fluctuations. Neither do these loose groupings suggest any seasonal effect on activity similar to that exerted on reproduction, for they occurred in September, June and December. The curves further show that little or no correlation can be made between the fluctuations in activity and the weight of the animal or the food consumption. In some cases the curves of activity and food intake correspond, but the evidence of cause and effect is not convincing. The noticeable increase in weight of A1, at the end of the curve, was due to the rapid growth of a tumor which finally caused its death.

In order to show more definitely the tendency toward three long fluctuations in activity approximately 200 days apart, which was suggested by the individual curves in figure 2 and table 2, composite curves representing the averages of these five rats were constructed. Figure 3 represents these average curves. The curve of average activity in this figure shows three fairly definite long fluctuations on which are superimposed other smaller elevations. The lack of regularity is doubtless due to the average representing too few individuals. This brings out the important fact, which we have often tried to make clear, that, due to the great individual

Fig. 2. Curves of activity (continuous line), daily food consumption (dotted line), and body weight (dash line) of the five virgin rats, A, B, C, D and E in experiment 2.

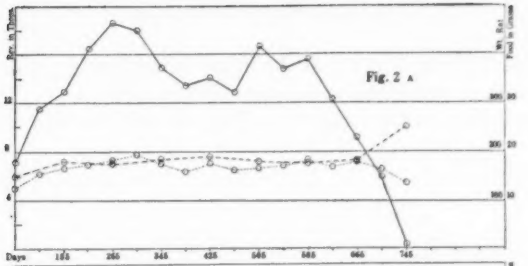
Fig. 2 A
Days

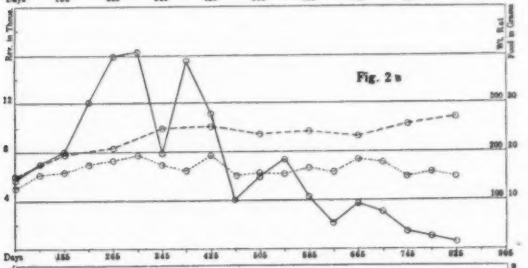
Fig. 2 B
Days

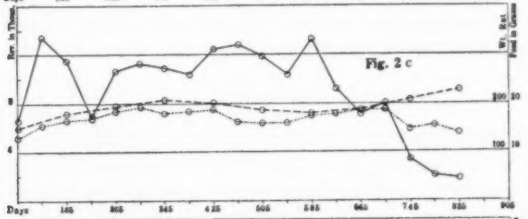
Fig. 2 C
Days

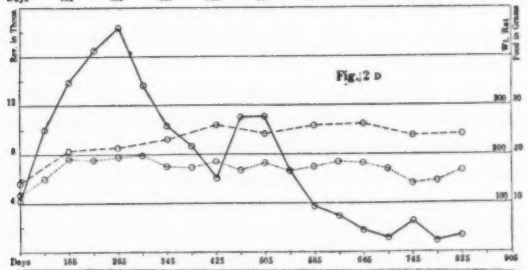
Fig. 2 D
Days

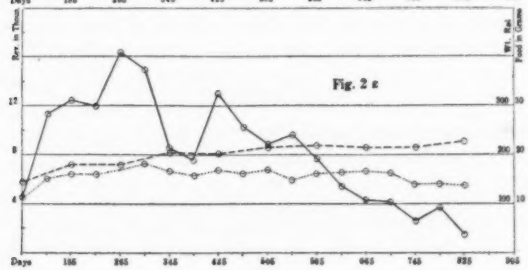
Fig. 2 E
Days

variation which obtains in the albino rat, a large number of animals must be used to obtain consistent and dependable results.

These data seem to show that the albino rat exhibits at least three long

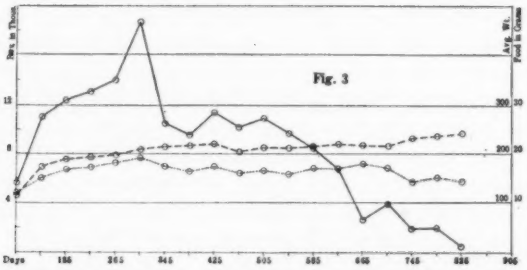

Fig. 3. Average curves of activity (continuous line), daily food consumption (dotted line), and body weight (dash line) of the five virgin rats of experiment 2.

fluctuations in activity, which are 200 or more days in length, and that on these long fluctuations are smaller ones of shorter duration, which vary in number and length in different animals.

SUMMARY

1. The female albino rat not only exhibits variations in daily activity, known as the oestrual rhythm, but also shows gradual fluctuations in average daily activity which extend over long periods of time, 200 days or more, and on these are superimposed shorter fluctuations.

2. These fluctuations in average daily activity are common to both sexes.

3. The lack of synchronism of these fluctuations in a group of animals indicates that changes in the environment, such as moisture, temperature, or barometric variations, are not the cause.

4. A greater tendency toward synchronous fluctuations existed in the males than in the females.

5. The fluctuations persisted in females after they had passed the menopause, and in senile males.

6. These fluctuations in activity appear to be of normal occurrence and must be considered in experiments dealing with activity.

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THE MECHANISM OF PARADOXICAL PUPIL DILATATION AND CONSTRICTION

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Historical. Anderson (1903b) after reviewing the literature found that following lesions of the efferent pathway the mechanism of paradoxical pupil dilatation consists essentially of increased muscle irritability. Lewandowsky (1900) who first used adrenalin to elicit paradoxical dilatation (1899), had previously arrived at the same conclusion. This view though true in general is unsatisfactory because it lacks specificity as to detail and offers no explanation for the varying periods of incubation observed after different experimental lesions, e.g., removal of the superior cervical ganglion (about 24 hours), section of the cervical sympathetic nerve (about 5 to 8 days) etc. Langendorff (1900) and Langley (1901) apparently sensed these difficulties for they believed that the period of incubation following lesions of the efferent dilator paths was in some way related to degeneration of the nerve elements.

Methods. Cats were used, each animal being carefully selected after exclusion of pupil abnormalities by inspection and an intravenous injection of adrenalin. Unilateral lesions of the afferent dilator paths were then made at various "levels" from the long ciliary nerves up to the brainstem. After the lesion each animal was studied at short intervals to ascertain the time that had to elapse before paradoxical dilatation could be elicited by adrenalin intravenously. In order to determine whether degeneration incidental to the lesion, or mere failure of conduction over the efferent dilator paths, was the essential mechanistic factor the problem of temporarily blocking these paths, and keeping them blocked for 24 hours, presented itself. After many more or less successful attempts it was found that by lightly packing the bulla tympani with absorbent cotton soaked in a 5 per cent solution of cocaine hydrochloride the passage of efferent dilator impulses could be effectively held in check for 24 hours. After dipping it in the cocaine solution the cotton must be squeezed almost dry as the solution in excess may reach the general circulation and dilate the pupils. With the proper amount of solution the pupils do not dilate and soon after the cotton has been packed into the bulla the homolateral pupil becomes smaller than its fellow. The cotton should be

changed 2 or 3 times within the 24 hours. The lesions were verified at autopsy.

Experimental facts. After the following lesions paradoxical dilatation was evoked by adrenalin intravenously as follows: 1. Section or injury of long ciliary nerves, in 24 hours (6 animals.) 2. Removal of superior cervical ganglion, in 23 to 24 hours (12 animals). 3. Continued cocainization, through the bulla tympani, of post ganglionic paths, in 23 to 24 hours (6 animals). After removal of the cocaine soaked cotton and irrigation of the bulla with normal salt solution the hypersensitivity of the dilator effectors disappeared within 24 hours. 4. Section of sympathetic, *a*, high in the neck, in 5 to 7 days though occasionally in 2 or 3 days in excitable animals (12 animals); *b*, low in the neck, in 8 to 10 days (6 animals). 5. Removal of stellate ganglion, in 9 to 11 days (3 animals). 6. Hemitransection of spinal cord, *a*, midway between nerve-roots C VII and VIII, in 12 to 14 days (6 animals) *b*, between roots C V and VI, in 15 days (6 animals) *c*, between roots C i and ii, in 15 to 17 days (8 animals). 7. Hemitransection of brain stem 0.5 cm. above *calamus scriptorius*, in 23 to 24 days (2 animals). In 6 animals after hemitransection of cord at or below the 3rd thoracic segment paradoxical dilatation was only irregularly and inconsistently demonstrable. In the regeneration period, i.e., about 12 to 16 weeks after vago-sympathetic section the dilator effectors lost their hypersensitiveness for adrenalin, etc., before excitability (conductivity) had returned for a fairly strong the faradic current (coil at 10.0 cm.) with the electrodes applied proximal to the site of section (3 animals).

After cord transection in the upper, middle and lower cervical region electrical stimulation of the sciatic nerve failed to evoke immediate dilatation in either pupil. Dilatation phenomena which appeared about 8 to 15 seconds after onset of stimulation (occasionally in 3 or 4 seconds) was frequently observed especially in young animals.

Discussion. As effector sensitization persists after complete degeneration of the long ciliary nerves and as paradoxical phenomena, may still be elicited after ergotoxine in dosage sufficient to abolish the pressor effects of adrenalin, the site of sensitization must be the myoneural junctions and chiefly, though not exclusively, towards the neural as opposed to muscular components. The sensitization observed after 24 hours of mild cocainization of the post-ganglionic paths, and the return to normal within 24 hours after discontinuance of the cocainization, point to conduction block, as the primary fundamental factor in the mechanism. Degeneration of the neurones, pre- or post-ganglionic, is therefore apparently not an essential factor. But since the efferent dilator impulses themselves are normally conditioned reflexly lesion or conduction block in the afferent dilator paths (affective system) by reducing the flow of efferent impulses

also induces effector sensitization. Compare Byrne (1921). During the regenerating period after section of one vago-sympathetic the disappearance of the effector hypersensitiveness before the return of excitability (conductivity) for the faradic current points to two separate sets of fibers in the cervical sympathetic one for the mediation of normal (reflex) dilator impulses and another, older, more resistant and endowed with greater regenerating power, for the mediation of the impulses fundamentally concerned in the mechanism of effector sensitization. There is, moreover, evidence tending to show that the latter impulses differ from the former in mode of initiation and perhaps of propagation. Thus after section of the cervical spinal cord although sciatic stimulation no longer elicits primary neural (reflex) pupil dilatation yet, under these conditions, section or crushing of one brachial plexus, or of one sciatic nerve, induces the pseudo-paradoxical phenomenon in the related pupil all of which implies that impulses other than those mediating reflex dilatation pass to the pupil from the injured primary affective neurones via Budge's inferior ciliospinal center and the cervical sympathetic nerve. Compare Byrne (1922b).

The efferent impulses concerned in the mechanism appear to be inhibitory since interception or reduction of them manifestly releases the related effectors permitting them to revert, wholly or in part, to the more primitive form of chemical as opposed to neural activation. Chemical effector activation has been insisted upon as a fundamental factor in the mechanism of all tropistic reactions by Loeb (1902) who seems to have shown conclusively that in many of the lower forms purposeful "reflex" movements can be executed after removal of the central nervous system. Indeed, in the mechanism of movements of any kind, whether spontaneous, coördinated or purposeful, the only property which Loeb concedes to the central nervous system is that of highly specialized conductivity. Be this as it may, it seems that the first step towards ordered neural functioning, on the motor as well as on the sensory side, is a certain degree of inhibition of the more primitive modes of functioning. Compare the reversion (protopathic dissociation) observed during the regeneration period after section of a peripheral sensory nerve (Rivers and Head 1908), (Byrne 1923a). As recovery progresses the affective system is partially inhibited by the critical so that its reactions instead of being maximal become graded. And as hyperfunctioning (hypermetabolism) of the primary affective neurones is a fundamental factor (Byrne 1922, 1924) in the mechanism of pseudo-paradoxical pupil dilatation as in that of protopathic dissociation, it seems, in the last analysis, that the inhibition exerted by the critical upon the affective system represents in effect a restraining influence exerted by higher more specialized neural mechanisms upon chemical processes taking place in the primary affective neurones and more

especially in the bodies of these neurones lying within the dorsal root ganglia (neuro-chemical inhibition). In their capacity to elaborate and even store a reserve of the materials that are the immediate progenitors of the nerve impulse (kinetoplasm) the affective differ widely from the critical neurones. In primitive affective mechanisms in which the reaction tends to be maximal, i.e., according to the all or none principle, excessive elaboration and storage of "neuro-potential" has practical significance whereas in critical mechanisms such excessive elaboration would be a hindrance rather than a help to the normal graded response.

In mechanism paradoxical constriction closely resembles paradoxical dilatation (Anderson, 1905) the only outstanding difference being the absence, in paradoxical constriction, of a definite incubation period. This is due presumably to the type of metabolic endowment of the neurones, efferent and afferent, involved in this typically critical mechanism. In paradoxical dilatation not only the afferent, but also the efferent neurones (chiefly the neurone bodies) are capable of discharging impulses for some time after lesion of their processes or of their preganglionic fibers. This capacity for after discharge seems to be the fundamental factor determining the presence and duration of the incubation period. Compare Langley (1911). And as the neurones, afferent and efferent, of critical mechanisms have little or no capacity for after discharge the flow of efferent (inhibitory) impulses to the constrictor effectors becomes at once so blocked or reduced after experimental lesions that paradoxical constriction soon manifests itself "spontaneously" or is immediately demonstrable by conjunctival instillation of pilocarpine. In operations or injuries of the skull in which the cranial nerves, more especially the oculo-motor or vestibular, have been injured the related pupil frequently exhibits "spontaneous" paradoxical constriction before or after death. On the other hand as the afferent and efferent neurones in affective mechanisms continue to discharge impulses for some time after the experimental lesion the sensitization of the dilator effectors can only be demonstrated after the lapse of a shorter or longer interval depending upon circumstances. The shorter average incubation period (6 to 8 days) (Byrne, 1922) after injury of the brachial plexus etc., as compared with 10 to 12 days after sciatic section (Byrne, 1921) seems to bear some relation to the higher degree of specialization of function (critical mechanisms) that obtains in the cat's fore limbs. The lower degree of specialized function in the hind limbs points to a relative predominance in these of affective mechanisms capable of prolonged after discharge. With the exception noted concerning the incubation period, the mechanism of paradoxical constriction so closely resembles that of paradoxical dilatation that what has been established for the latter applies equally to the former.

As the afferent paths mediating reflex pupil dilatation pass all the way

to the upper medulla before impinging upon the efferent dilator neurones, sciatic stimulation, after cervical cord transection, no longer evokes primary neural dilatation but only a secondary delayed form which usually occurs in from 8 to 15 seconds after onset of stimulation. This dilatation is effected by an intermediate (neuro-chemical) form of activation which stands midway developmentally between chemical (myo-neural, tropistic) and purely neural (reflex) activation. As it seems to occur only in association with muscular contractions chiefly in the flexors of the hind limbs or of the trunk or both, this form of activation seems to be conditioned by the presence in the blood stream of substances (e.g., sarcolactic acid) accumulated during muscular contraction. Because of their failure to measure the latency periods it seems probable that it was this form of activation and not primary neural (reflex) dilatation that Luchsinger (1880), Guillebeau and Luchsinger (1883) and Anderson (1903a) obtained upon sciatic stimulation after transection of the cervical spinal cord. The failure of Chauveau (1861) and Kowalewsky (1886) to obtain reflex dilatation phenomena can be accounted for by the fact that even in young animals after the spinal shock from the cervical cord transection has passed off sciatic stimulation may not, for one cause or another, induce the necessary muscular contractions. In cases like these the author frequently found that rest, especially when combined with intravenous administration of glucose and adrenalin, restored the muscle irritability so that sciatic stimulation evoked muscular contraction and the associated pupil dilatation phenomena. The intermediate (neuro-chemical) form of activation takes place primarily in Budge's cilio-spinal center and secondarily in the superior cervical ganglion whereas purely chemical activation occurs chiefly at the myo-neural junctions (dilator effectors). This is shown by the fact that after cord transection at or above root C 1 and section of one cervical or vago-sympathetic, or removal of one superior cervical ganglion, stimulation of the sciatic evokes, after a few seconds in young animals especially, well-marked dilatation phenomena (in pupil, membrane and palpebral fissure) on the side with intact cervical sympathetic, the phenomena appearing on the side with cut sympathetic only many seconds later and still later in cases where the superior cervical ganglion had been removed.

The progressive lengthening of the incubation period for paradoxical dilatation, varying from 24 hours to 24 days, as the lesion recedes from the long ciliary nerves toward the brain-stem, seems to be due to the presence of collections of neurone bodies at various "levels" along the course of the efferent dilator paths in the brain-stem, cervical cord and cervical sympathetic. Some of these collections are large as in the superior cervical ganglion, and the hypothalmic center of Karplus and Kreidl (1909), (1910) whilst others are of microscopic size. There is anatomical evidence

of small collections amongst the post-ganglionic fibers, e.g., in plexus ciliaris found in the ciliary body adjoining the iris (Spalteholtz, 1906). Compare Ewart (1890) who found that cells from the ciliary ganglion had wandered a considerable distance along the ciliary nerves towards the eyeball. Langley (1898) concludes "that all the fibers which pass to the cervical sympathetic from the spinal cord reach the nerve without having nerve cells on their course" but in a preceding paragraph he admits that "a few of the nerve cells of the superior cervical ganglion occasionally occur in the trunk of the nerve." The work of Langley and Dickinson

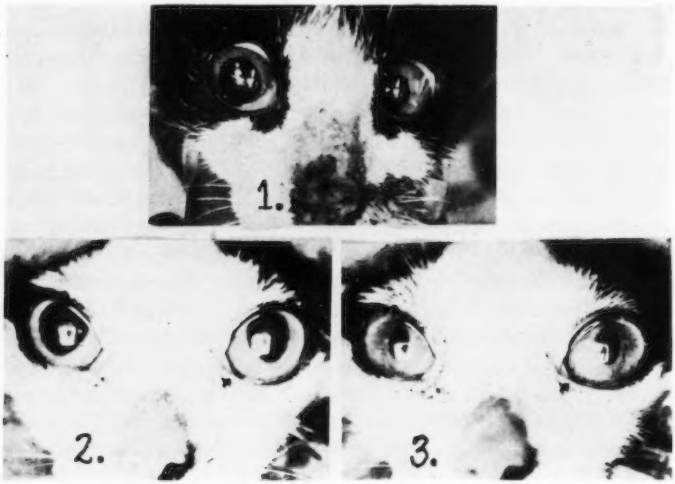

Fig. 1. Paradoxical dilatation in right eye evoked by adrenalin instillation after 23¾ hours of mild cocainization of post-ganglionic fibers through bulla tympani; sensitization passed off within 24 hours after discontinuing cocainization.

Fig. 2. Paradoxical dilatation in right eye evoked by adrenalin intravenously on 17th day after right hemitransection of cord between roots C IV and V.

Fig. 3. Same animal as in figure 2 before adrenalin injection.

(1889, 1890) although it apparently tends to disprove the existence of relay stations in the course of the cervical sympathetic nerve by no means actually does so.

The most plausible explanation of the lengthening of the incubation period as the lesion recedes from the periphery is that the groups of neurone bodies strewn along the course of the efferent dilator pathway continue to discharge impulses for some time after severance or injury of their preganglionic fibers. This seems to be substantiated by an experiment originally performed by Budge (1855), and since verified by numerous

investigators. After removal of the superior cervical ganglion on one side and simultaneous section of the cervical sympathetic nerve on the opposite side, the pupil on the ganglionectomized side is smaller than that on the side of the sympathetic section and remains so until the superven-tion, in 24 hours, of paradoxical dilatation when it becomes the larger pupil. This view is further supported by the fact observed by Byrne and Sherwin (1924) that operative procedures capable of inducing spinal shock, in which neural function tends to be depressed or temporarily suspended, can considerably shorten the period of incubation after superior ganglionect-omy or section of the cervical sympathetic.

In hemitransections of the cord at and below the 3rd thoracic segment it was found that true paradoxical dilatation was only irregularly and inconsistently demonstrable. The results in these cases stood in sharp contrast with those observed after lesions of the sciatic nerve, brachial plexus and posterior nerve roots. Apparently they are to be accounted for by the fact that the affective paths in their course to the brain are bilateral, traversing a series of short relays (Ranson and Hess, 1915) which cross and recross. Compare also Ranson and Billingsley (1916, 1917). The main objective of the dilator (affective) paths as they lie in the peripheral nerves and nerve-roots is the cilio-spinal center upon which they ultimately impinge directly, or indirectly via the upper medullary and hypothalmic centers. Interruption of functional continuity be-tween these paths and the efferent dilator sympathetic neurones is the fundamental factor in the mechanism of paradoxical dilatation following lesions of the afferent paths. Compare Byrne (1921).

The demonstration of true paradoxical dilatation after hemitransection of the cervical cord and brain-stem was not so outspoken as one might expect considering the fact that in these regions the lesion supposedly severs the efferent dilator paths. During the experiments this difficulty was fully appreciated and recourse was had to a variety of experimental procedures to insure completeness, etc., of the hemitransections. After prolonged study, from day to day, of the effects of the lesions upon the pupils and membranes, coupled with verification of the lesions at autopsy, the conclusion seemed inevitable that the efferent dilator (cilio-spinal) paths in the cervical cord undergo partial decussation. Upon no other hypothesis did it seem possible to explain the phenomena observed. This decussation occurs probably in bulk at or near the level of Budge's cilio-spinal center. In any event the pupil inequality, etc., observed indicate that about 40 per cent of the efferent dilator paths proper cross to the opposite side, the percentage being somewhat larger for the paths related to membrane withdrawal. Compare Karplus and Kreidl (1909). The small preponderance of efferent uncrossed paths accounts satisfac-torily for the paradoxical dilatation phenomena demonstrable in the

homolateral eye after hemitransection of the cervical cord and brain-stem.

CONCLUSIONS

1. The fundamental factor in the mechanism of effector sensitization is conduction block in the efferent paths, or its equivalent on the afferent side, whereby the flow of efferent inhibitory impulses to the dilator or constrictor effectors is completely or partially interrupted.

2. As a secondary factor in the mechanism the conduction block affects the myoneural junctions permitting them to revert to the more primitive mode of chemical, as opposed to neural or neuro-chemical effector activation.

3. The more or less well defined period of incubation observed in paradoxical dilatation is determined by the capacity of the injured afferent or of the decentralized efferent neurones to continue to discharge impulses which partially inhibit chemical effector activation.

4. The absence of a definite incubation period in paradoxical constriction is to be attributed to the fact that the injured afferent or the decentralized efferent neurones are incapable, after the experimental lesion, of discharging impulses which for a time might inhibit chemical effector activation.

5. The efferent dilator (cilio-spinal) paths decussate partially at or near the level of Budge's lower cilio-spinal center.

6. There is evidence of two separate sets of fibers in the cervical sympathetic one for the mediation of normal pupil dilator impulses and the other for the mediation of impulses that partially inhibit chemical effector activation.

7. An intermediate (neuro-chemical) form of activation stands midway developmentally between chemical, (myo-neural, tropistic) and purely neural (reflex) activation.

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INCREASED EFFICACY OF SUBCUTANEOUS WHEN COMPARED WITH INTRAPERITONEAL ADMINISTRATION OF THE OVARIAN HORMONE

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Effects are secured by the administration of a smaller total amount of ovarian hormone when the dosage is not given at one time but is divided and administered once daily several times. This is in accord with the findings of Allen and Doisey and co-workers. We may illustrate it with work in which we used alcoholic extracts of whole pig ovaries prepared according to the above workers.[1] Two spayed female rats (GH 2313 and B 2288) were each given single subcutaneous injections of 50 mgm. of the above product without effect; two other animals (BH 2532 and BH 2347) were then given single doses of 200 mgm., again without effect.[2] At length when the single skin dose was as high as 300 mgm. the cornified cells of oestrus were secured (BH 2301; also with 350 mgm.—BH 2516). When 200 mgm. were given each morning for two days this total (200 mgm.) evoked typical cornification; indeed, 100 mgm. given in two daily 50-mgm. injections were completely efficacious.

These results pointed the way to the explanation of an early failure to secure the cornified oestrous smear with single very large intraperitoneal injections of this hormone. Two things remained to be done; first, the intraperitoneal method had to be tried by daily small doses and, secondly, the minimum effective dosage done in this way had to be compared with the same dosage and even lower dosage by the subcutaneous route. Two animals (B 2525 and B 2124) were injected daily intraperitoneally with 25 mgm. of our substance. In the case of the first animal (B 2525) four such injections were made; in the second (B 2124) six daily injections were done; but in neither case was the typical oestrous cornified smear obtained (table 1).

When now in two animals (GH 2305 and GH 2222) 50 mgm. were administered daily intraperitoneally for four days cornified cells began to appear in both cases on the fourth day and were abundant on the fifth day (table 2).

[1] Journ. Biol. Chem., 1924, lxi, 711.

[2] The large typical cornified cells did not appear though leucocytes disappeared from the vaginal smear.

TABLE 1

DESIGNATION OF RAT	DATE SPAYED	DATE OF INJECTION	DOSE OF OVARIAN EXTRACT	METHOD OF INJECTION	VAGINAL CELL SMEAR
			mgm.		
B 2525	11–27–25	2–11–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–12–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–13–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–14–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–15–26	None	Not injected	Leucocytes and epithelial cells
		2–16–26	None	Not injected	Leucocytes and epithelial cells
		2–17–26	None	Not injected	Leucocytes and epithelial cells
B 2124	1–22–26	2–11–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–12–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–13–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–14–26	25	Intraperitoneal	Epithelial cells only
		2–15–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–16–26	25	Intraperitoneal	Leucocytes and epithelial cells
		2–17–26	None	Not injected	Leucocytes and epithelial cells

TABLE 2

DESIGNATION OF RAT	DATE SPAYED	DATE OF INJECTION	DOSE OF OVARIAN EXTRACT	METHOD OF INJECTION	VAGINAL CELL SMEAR
			mgm.		
GH 2305	11–25–25	2–11–26	50	Intraperitoneal	Leucocytes and epithelial cells
		2–12–26	50	Intraperitoneal	Leucocytes and epithelial cells
		2–13–26	50	Intraperitoneal	Leucocytes and epithelial cells (few cornified)
		2–14–26	50	Intraperitoneal	Leucocytes and epithelial cells (few cornified)
		2–15–26	None	Not injected	Cornified cells and leucocytes
		2–16–26	None	Not injected	Leucocytes and epithelial cells
		2–17–26	None	Not injected	Leucocytes and epithelial cells
GH 2222	1–19–26	2–11–26	50	Intraperitoneal	Leucocytes and epithelial cells
		2–12–26	50	Intraperitoneal	Leucocytes and epithelial cells
		2–13–26	50	Intraperitoneal	Leucocytes and epithelial cells
		2–14–26	50	Intraperitoneal	Leucocytes and epithelial cells (some cornified)
		2–15–26	None	Not injected	Cornified cells only
		2–16–26	None	Not injected	Leucocytes and epithelial cells
		2–17–26	None	Not injected	Leucocytes and epithelial cells

TABLE 3

DESIGNATION OF RAT	DATE SPAYED	DATE OF INJECTION	DOSE OF OVARIAN EXTRACT	METHOD OF INJECTION	VAGINAL CELL SMEAR
			mgm.		
BH 2146	11-27-25	2-11-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-12-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-13-26	25	Subcutaneously	Leucocytes, epithelial and cornified cells
		2-14-26	None	Not injected	Cornified cells only
		2-15-26	None	Not injected	Cornified cells only
		2-16-26	None	Not injected	Leucocytes and epithelial cells
BH 2275	11-27-25	2-11-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-12-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-13-26	25	Subcutaneously	Epithelial and cornified cells
		2-14-26	None	Not injected	Cornified cells only
		2-15-26	None	Not injected	Cornified cells and leucocytes
		2-16-26	None	Not injected	Leucocytes and epithelial cells

TABLE 4

DESIGNATION OF RAT	DATE SPAYED	DATE OF INJECTION	DOSE OF OVARIAN HORMONE	METHOD OF INJECTION	VAGINAL CELL SMEAR
			mgm.		
W 2223	11-27-25	2-17-26	25	Intraperitoneal	Leucocytes and epithelial cells
		2-18-26	25	Intraperitoneal	Leucocytes and epithelial cells
		2-19-26	25	Intraperitoneal	Leucocytes and epithelial cells
		2-20-26	None	Not injected	Leucocytes and epithelial cells
BH 2286	11-27-25	2-17-26	25	Intraperitoneal	Leucocytes and epithelial cells
		2-18-26	25	Intraperitoneal	Leucocytes and epithelial cells
		2-19-26	25	Intraperitoneal	Leucocytes and epithelial cells
		2-20-26	None	Not injected	Leucocytes and epithelial cells
* *	* *	*	*	* * *	* * * * * * * *
BH 2584	11-27-25	2-17-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-18-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-19-26	25	Subcutaneously	Epithelial cells only. Mated
		2-20-26	None	Not injected	No plug; no sperm; cornified cells
		2-21-26	None	Not injected	Cornified cells
		2-22-26	None	Not injected	Leucocytes and epithelial cells
BH 2187	11-27-25	2-17-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-18-26	25	Subcutaneously	Leucocytes and epithelial cells
		2-19-26	25	Subcutaneously	Epithelial cells only. Mated
		2-20-26	None	Not injected	No plug; no sperm; cornified cells
		2-21-26	None	Not injected	Cornified cells
		2-22-26	None	Not injected	Leucocytes and epithelial cells

This then was the minimum effective daily intraperitoneal dose; it was administered subcutaneously to an animal (W 2284) for three days, cornified cells appearing on the third day. We then administered to two animals (BH 2146 and BH 2275) daily subcutaneously for three days 25 mgm. of the substance, an amount proven insufficient by the intraperitoneal route even with six injections. In both cases cornified elements were present by the third day and continued for two days thereafter (table 3).

For further confirmation, the work was carefully repeated at the 25-mgm. level and, as table 4 will show, at this level of dosage the subcutaneous administration is always effective and intraperitoneal administration ineffective.

RECIPROCAL REACTION AS A POSSIBLE LOCAL MECHANISM IN THE CAT

I. The Reciprocal Reaction of the Gastrocnemius and the Tibialis Anterior

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Sometime ago (Stein and Tulgan, 1925) we reported a series of experiments on the question of reciprocal reaction of antagonistic muscles in the frog. The muscles experimented on were the gastrocnemius and the tibialis anterior. After many experiments in which portions of the nervous system were eliminated in sequence, we found that direct stimulation of the gastrocnemius electrically caused it to contract and at the same time caused the tibialis anterior to relax synchronously with the contraction of the gastrocnemius. The same phenomenon occurred after the leg was amputated above the knee-joint and all the muscles and nerves of the thigh had been removed. Therefore the reaction that occurred here must necessarily have been of a local character.

The phenomenon was further studied after injecting novocaine into the knee-joint and into the connective tissue surrounding this joint and the tendonous origins of the muscles. This resulted in a cessation of the relaxation of the tibialis anterior, but the gastrocnemius contracted as before when directly stimulated electrically. However, direct stimulation, electrically, of the tibialis anterior, caused it to contract, indicating that the muscle could function and that the novocaine must have paralysed some local nervous mechanism probably present either in the knee-joint or in the connective tissue around the joint or the tendonous origins of the muscles. This mechanism which controlled the reciprocal action of the two muscles in question must have been of a nervous character since novocaine does not act directly on muscle, but upon nerve tissue. The possible stimulation of nerves of this local mechanism was avoided by not placing the electrodes too close to the tendonous orgin of the gastrocnemius.

From the results of these experiments we came to the following conclusions:

1. At the knee-joint of the frog there is a local mechanism which is

concerned with the reciprocal reaction of the gastrocnemius and the tibialis anterior muscles.

2. This mechanism is probably subsidiary to a higher mechanism in the central system.

3. This mechanism is of a local type since the injection of novocaine in and about the joint abolishes it.

4. The reciprocal reaction of these two muscles could not be reversed.

In the present study cats were employed. The gastrocnemius and the tibialis anterior were dissected out up to their tendons of origin. Their tendons of insertion were connected by means of pieces of string which lead over sensitive pulleys to two muscle levers, one for each of the muscles. The lever to which the tendon of the gastrocnemius was attached was after-loaded with a twenty-gram weight. The lever to which the tendon of the tibialis anterior was attached was not after-loaded, but loaded by the weight of the lever and twenty grams. This was necessary in order to have the proper tension for the muscle. Fine copper wires were used as electrodes. One was tied around the tendonous insertion of the gastrocnemius and the other was inserted directly into the muscle near its tendonous origin. The two muscles in question were so arranged that their origins and their fleshy parts were sufficiently separated so that the action of the protagonistic muscle did not affect mechanically the antagonistic muscle. Every precaution was taken to fix the limb in such a position, so that the muscles in question might function properly without mechanically affecting the limb itself. In other words the muscles acted from a fixed point. We endeavored to eliminate as far as possible any artefacts which might affect the results. The limb was immobilized in such a manner that there was as little interference as possible with the blood and nerve supply to it. When the protagonist contracted and the antagonist relaxed one of us observed carefully the contracting and relaxing muscles, to be sure that no movement occurred other than that of the muscles, while the other recorded the tracings on a rapidly moving drum. The stimulus used was an optimum tetanizing current. An optimum stimulus was found necessary to obtain the results we are reporting. With too weak a current, the antagonist did not relax, and with too strong a current not only both muscles contracted, but also the other muscles of the leg. This was probably due to a spreading of the current to the other muscles.

One of the most important factors in this experiment was the maintenance of the proper degree of tension on the two muscles. This correlated with the proper strength of stimulus gave us the results described below.

After several records had been made the antagonist was stimulated directly in order to make certain that it was functioning. Notwith-

standing the exposure and the trauma to which these muscles were subjected, irritability persisted for a long time.

The experiment was performed under the following conditions:

1. The gastrocnemius was stimulated with the entire nervous system intact.

2. The sciatic and femoral nerves were sectioned as high in the thigh as possible and the gastrocnemius directly stimulated.

3. Novocaine and adrenalin were injected into the joint, the connective tissue about the joint and the tendo-muscular origins of the protagonistic and antagonistic muscles.

EXPERIMENTAL RESULTS. 1. *Stimulation of the gastrocnemius with the entire nervous system intact.* Direct stimulation of the gastrocnemius caused its contraction and a synchronous relaxation of the tibialis anterior.

2. *The sciatic and femoral nerves sectioned as high in the thigh as possible.* This was done in order to eliminate as far as possible activity on the part of the central nervous system affecting this phenomenon. The peripheral portions of these nerves were left intact. Direct stimulation of the gastrocnemius still gave the same results, showing that the central nervous system was not wholly responsible for this reciprocal reaction, if at all.

3. *Novocaine and adrenalin injected into the joint, into the connective tissue surrounding the joint and the tendo-muscular origins of the two muscles.* Some time after the injection of the novocaine and adrenalin there was a gradual cessation of relaxation of the tibialis anterior when the gastrocnemius was stimulated, although the latter muscle contracted as before. Finally, the relaxation of the tibialis anterior ceased entirely when the gastrocnemius was stimulated. This phenomenon we were able to demonstrate with a fair degree of consistency.

DISCUSSION. Twenty experiments were performed on cats and numerous tracings of our results were obtained. The published tracings are characteristic. Figure 1 shows the reciprocal reactions of the two muscles when the gastrocnemius was directly stimulated with the sciatic and femoral nerves intact. The same phenomenon was observed, as is shown in figure 1, although the sciatic and femoral nerves had been cut. Figure 2 shows the effect of novocaine on the reciprocal actions of these muscles.

It will be obvious that the direct stimulation of the gastrocnemius in the first two cases causes a relaxation of the tibialis anterior, whereas in the third case the relaxation of the tibialis anterior did not occur. It appears therefore that the injection of the novocaine in and about the knee-joint paralyzed some nervous mechanism present in that locality which controls the reciprocal reaction of the two muscles, when the gastrocnemius was directly stimulated. The inhibition of relaxation

came on gradually until finally the relaxation of the tibialis anterior was completely abolished.

We believe this indicates the presence of some nervous mechanism within or closely surrounding the knee-joint which may be responsible for the reciprocal action of these two muscles. We also believe it is a nervous mechanism, since it is known that novocaine abolishes irritability and conductivity of nerves, but does not affect muscles.

We could not reverse the reaction in this series of experiments. By reverse action is meant that when the tibialis anterior is stimulated directly it was impossible for us to observe or record a relaxation of the gastrocnemius, although experiments now being conducted by us with

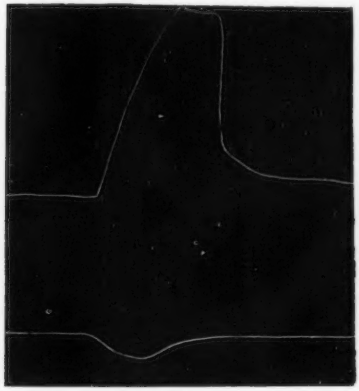

Fig. 1

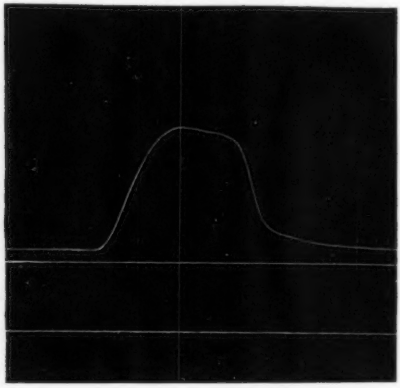

Fig. 2

Fig. 1. Reciprocal reaction of the gastrocnemius and tibialis anterior with the femoral and sciatic nerves intact.

Fig. 2. The effect of novocaine on the reciprocal reaction of the gastrocnemius and tibialis anterior muscles.

groups of antagonistic muscles in other parts of the body show that this antagonistic reaction may be reversed.

We cannot explain the non-reversal of this reaction in this case and its obtainment in experiments with other groups of antagonistic muscles. We were also unable to record or observe the relaxation of the gastrocnemius when the tibialis anterior was directly stimulated in frogs.

Is it not possible that there is a local mechanism to aid greatly flexion in the lower extremities of these animals which use flexion more frequently than extension? May this not be a mechanism of adaptation both in the frog and the cat to facilitate more rapid function? We have found a local mechanism in or about the knee-joint of a mammal, the cat, which resembles the mechanism found by us in the frog. We think that this

local mechanism is probably subsidiary to a similar mechanism located in the central nervous system and that this local mechanism may be stimulated or inhibited by the one in the central nervous system.

Is it not possible that this is a local mechanism facilitating more rapid function, thus enabling the animal to perform quickly the acts of jumping, sitting, etc., and assisting greatly reflex actions, and probably diminishing the perceptibility of these actions?

These experiments prove that such local mechanisms may exist in mammals and suggests that these local mechanisms assist in a great many of our own actions.

Further work on other groups of antagonistic muscles is in progress.

CONCLUSIONS

1. At the knee-joint of the cat there is a local mechanism concerned with the reciprocal reaction of the gastrocnemius and the tibialis anterior muscles.

2. The mechanim is probably subsidiary to a higher mechanism in the central nervous system.

3. This local mechanism is of a nervous type since the injection of novocaine in and about the knee-joint abolishes it.

4. The reciprocal reaction of the two muscles could not be reversed.

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STUDIES OF THE THYROID APPARATUS

XXXIII. The Rôle of the Thyroid Apparatus in the Growth of the Reproductive System

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The significance of the thyroid apparatus at different stages of development in the growth of the body and of the central nervous system of the albino rat has been discussed in the preceding four papers (1), (2), (3), (4). The present report deals with the growth of the testis, ovary, epididymis and uterus of the same rats from the same point of view. It may be recalled that the data were obtained from rats which had been allowed to grow until 150 days old, after thyroid or parathyroid removal at 23, 30 50, 65, 75 and 100 days of age, and their controls. For reasons and with limitations given elsewhere (1) the growth sequelae of thyro-parathyroidectomy are interpreted in terms of thyroid deficiency alone.

Since a point of view of these studies is that of differential development it is well to have in mind the normal course of events, with which the experimental material may be compared. This is obtained from the absolute daily percentage increment values for the successive periods used in the study. The figures, computed from the raw data in table 2, are given in table 1. For comparison Hatai's (5), (6) data for the testis and epididymis, and Osterud's data on the ovary and uterus, all of which are found in Donaldson's book (7), are given.

It is seen that the maximum percentage rate of growth of the reproductive system of my series preceded those of Hatai and Osterud. This is due to the better nutritive and environmental conditions employed (8). It is known that a sub-optimal diet retards reproductive development in the female rat (9) (10). My data confirm these earlier findings and extend them to include the male. Many reports show that the human reaction is similar (11), (12), (13).

The maximum percentage rate of growth of the male gonads precedes that of the female. This is consistent with the fact that ripe spermatozoa (14) appear before ripe ova (15) in the albino rat. It is also consistent with the fact that sexual activity in the male (16) precedes that in the female (17). While dietary factors (18), (19) may affect the time of appearance

of these phenomena, it is probable that the order of sex difference is the same in any stock in which both sexes are kept under like conditions.

The maximum percentage rate of growth is an expression of a heightened formation of immature reproductive elements in the testis, and in the ovary of an increase in size but not in number of ova (15), preparatory to the attainment of sexual maturity. The lag exhibited in the case of the ovary is an expression of a later reaching of full maturity in that organ.

TABLE 1

The absolute percentage increment per day for each of the successive periods

Males

PERIOD	HAMMETT			HATAI		
	Testis	Epididymis	E/T	Testis	Epididymis	E/T
days						
23–30	19.91	10.64	0.53	8.14	7.74	0.95
30–50	12.17	9.94	0.82	12.80	10.85	0.85
50–65	2.06	5.42	2.63	7.60	8.17	1.08
65–75	2.03	4.16	2.05	2.70	7.41	2.74
75–100	0.68	2.91	4.29	1.11	2.41	2.16
100–150	0.45	1.51	3.36	0.36	0.58	1.61

Females

	HAMMETT			OSTERUD*		
	Ovary	Uterus	U/O	Ovary	Uterus	U/O
23–30	0.52	−1.23	−2.36	1.55	−0.97	−0.62
30–50	4.50	24.89	5.53	2.31	18.00	7.82
50–65	4.10	7.56	1.85			
65–75	3.36	3.13	0.93	7.94	3.19	0.40
75–100	0.90	2.21	2.49	0.62	3.74	6.04
100–150	0.21	0.64	3.07	0.50	0.94	1.34

* From Osterud's data the following periods approximate most closely those used in my study: From 26 to 33; from 33 to 61; from 61 to 81; from 81 to 94, and from 94 to 165 days of age.

Similar sex differences in differential development in water-solids relation in the central nervous system suggest a tentative basis for interpretation. In both gonads and central nervous system (4) the development of the male precedes that of the female. In both there is a characteristic lipoid (not fat) metabolism (20) (21). It is possible that the sex difference in order of gonad and central nervous system development is an expression of a differential influence of sex specific regulators on lipoid (not fat) metabolism; that sexual maturity is associated with the production of

an optimum concentration of these lipoids in the gonads, and that the male stimulus accelerates such production.

From Donaldson's records (7) it is seen that the male albino rat is heavier at birth than the female. Shortly thereafter the female becomes heavier. This reversal is maintained up to the age of 49 days, when the male again becomes heavier. These shifts are roughly associated in time with the sex differences in maximum gonad growth (table 1) and the associated phenomena. They may be related causally to gonad incretory development.

Sex hormone stimulation of activity has been demonstrated by Wang (22), Slonaker (23), (24) and Hoskins (25). The onset of puberty determines an increase in activity in both sexes. This is followed by a decrease in the male but not in the female (26). This indicates a persistence of heightened activity in the latter beyond that exhibited by the former. Wang (22), Hitchcock (27) and others have shown that sexually mature females are more active than sexually mature males. Perhaps the falling below of the growth curve of the male during the period of its active gonad growth, is due to an increased activity emanating from testicular incretory stimulation, and that, when the ovarian incretory stimulation comes into play at the somewhat later age, a similar absolute and relatively increased activity brings the growth of the female down to the normal sub-male relation. This interpretation is consistent with the report of Slonaker (26) and others that exercised rats are less heavy than less active animals.

Age shifts in sex difference in growth of man are similar in type. Whether they may be correlated with sex differences in gonad development is as yet undetermined.

As would be expected the period of maximum growth capacity of the epididymis and uterus coincides with that of their respective gonads. Consistent with this are the correlation coefficients in the adult rat. Both testis and epididymis, and ovary and uterus show a good degree of positive association. The value for the former is 0.839 ± 0.018, and for the latter 0.427 ± 0.050. These coefficients were computed from the weights of the organs removed from control rats 150 days of age (121 of each sex). Stabilization by the method of partial correlation for the general factors contributing to organ size as carried by the body weight, does not alter the sex difference, though it lowers the absolute values.

The weight association of the ovary and uterus is less than that of the testis and epididymis for the following reason. The periodic incretory activity of the ovary conditions a high variability in weight of the uterus, without a corresponding change in weight of the gonad. The coefficients of variability of the two organs show this. That for the ovary is 15.72 ± 0.70, that for the uterus is 28.78 ± 1.35. On the other hand there is no evidence that the incretory activity of the testis affects the variability

TABLE 2

The observed mean weights of the testes, ovaries, epididymis and uterus of the several groups

AGE SERIES	THYROPARATHYROIDECTOMIZED					PARATHYROIDECTOMIZED			
	At beginning*			At end (150 days)		At beginning*		At end (150 days)	
	Ref. controls	Controls	Tests	Controls	Tests	Controls	Tests	Controls	Tests
	Testes								
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
23	0.1264±0.0030	0.1337	0.1300	2.4702±0.0558	2.2428±0.0693	0.1297	0.1260	2.3398±0.1050	1.9516±0.0911
30	0.3026±0.0131	0.2961	0.2917	2.3836±0.0582	2.1460±0.0681	0.3005	0.2995	2.5095±0.0324	2.3334±0.0536
50	1.0393±0.0511	1.0117	1.0037	2.3850±0.0803	2.1825±0.0890	1.0175	0.9972	2.3716±0.0632	2.3229±0.0859
65	1.3605±0.1059	1.2946	1.3115	2.3012±0.0481	2.1989±0.0722	1.3171	1.3227	2.3622±0.0318	2.0545±0.0669
75	1.6362±0.0594	1.5679	1.5894	2.1930±0.0789	1.9689±0.0474	1.6016	1.5697	2.2253±0.0875	1.7489±0.1031
100	1.9139±0.0563	1.8877	1.8740	2.2546±0.0688	2.0240±0.0806	1.8877	1.8436	2.2546±0.0688	2.1546±0.0679
	Ovaries								
23	0.0164±0.0007	0.0159	0.0156	0.1012±0.0027	0.0682±0.0046	0.0164	0.0162	0.0975±0.0024	0.0799±0.0038
30	0.0170±0.0005	0.0167	0.0165	0.0885±0.0027	0.0663±0.0023	0.0170	0.0167	0.0948±0.0031	0.0781±0.0030
50	0.0323±0.0018	0.0311	0.0310	0.0961±0.0029	0.0538±0.0038	0.0322	0.0317	0.0897±0.0014	0.0708±0.0034
65	0.0521±0.0037	0.0507	0.0492	0.0930±0.0027	0.0700±0.0045	0.0509	0.0499	0.0921±0.0025	0.0718±0.0048
75	0.0696±0.0049	0.0674	0.0665	0.0975±0.0024	0.0654±0.0045	0.0661	0.0663	0.0907±0.0030	0.070 ±0.0045
100	0.0850±0.0033	0.0823	0.0823	0.0917±0.0049	0.0604±0.0012	0.0823	0.0811	0.0917±0.0049	0.0724±0.0044

Epididymis

23	0.0200±0.0008	0.0212	0.0206	0.8395±0.0190	0.7098±0.0238	0.0205	0.0199	0.8242±0.0291	0.6188±0.0328
30	0.0349±0.0017	0.0341	0.0336	0.8331±0.0219	0.6812±0.0214	0.0347	0.0345	0.8717±0.0118	0.7686±0.0252
50	0.1043±0.0055	0.1015	0.1007	0.8176±0.0266	0.7165±0.0326	0.1021	0.1001	0.8252±0.0218	0.7496±0.0247
65	0.1891±0.0220	0.1799	0.1823	0.7830±0.0190	0.6751±0.0252	0.1831	0.1839	0.8370±0.0135	0.6731±0.0286
75	0.2677±0.0186	0.2565	0.2600	0.7563±0.0301	0.6437±0.0480	0.2620	0.2568	0.7895±0.0342	0.5364±0.0374
100	0.4624±0.0220	0.4561	0.4528	0.7556±0.0227	0.6221±0.0310	0.4561	0.4454	0.7556±0.0227	0.6724±0.0340

Uterus

23	0.0163±0.0004	0.0158	0.0155	0.5239±0.0181	0.3958±0.0347	0.0163	0.0161	0.5497±0.0248	0.4486±0.0447
30	0.0149±0.0011	0.0146	0.0145	0.5242±0.0215	0.3679±0.0167	0.0149	0.0146	0.5305±0.0208	0.3774±0.0253
50	0.0891±0.0102	0.0857	0.0854	0.4985±0.0225	0.2838±0.0392	0.0889	0.0875	0.4316±0.0166	0.3356±0.0300
65	0.1901±0.0227	0.1849	0.1795	0.4634±0.0349	0.3481±0.0232	0.1858	0.1821	0.4565±0.0318	0.3639±0.0345
75	0.2495±0.0254	0.2417	0.2384	0.5484±0.0243	0.3283±0.0350	0.2371	0.2375	0.4697±0.0329	0.3187±0.0415
100	0.3773±0.0298	0.3654	0.3654	0.4934±0.0408	0.2354±0.0074	0.3654	0.3598	0.4934±0.0408	0.3205±0.0353

* The "beginning" values for controls and tests are calculated not observed values.

of the epididymis. The C.V. for the two is of the same order of magnitude. That for the testis is 13.80 ± 0.56, that for the epididymis is 15.89 ± 0.61. It is probable that this high degree of weight correlation between testis and epididymis is an expression of the storage in the latter of spermatozoa.

From the columns headed $\frac{\text{E (pididymis)}}{\text{T (estis)}}$ it is seen that during the two growth periods from 23 to 50 days of age the growth capacity of the testis is greater than that of the epididymis, while in all later periods the reverse is the case. This reversal is coincident with the first appearance of ripe spermatozoa and the awakening of copulatory ability. It is clear that the relatively greater growth of the testis is an expression of the prepubertal development of immature spermatozoa. This is organo-specific. With the onset of puberty storage occurs in the epididymis. This sudden addition to the weight of the organ gives rise to the relative acceleration of its percentage rate of growth to which an increased growth of the epididymis, because of the new demand, contributes.

The maintenance and even increase in the growth superiority of the epididymis in the later age periods in my series is due to a lack of opportunity for copulation, since the males were kept separated from the females from weaning on. This opinion is derived from the fact that in Hatai's series of general and presumably mated stock the E/T relation decreases sharply after the attainment of the maximum at the height of puberty.

From table 1 it is seen that the uterus underwent an actual shrinkage or loss of weight during the first period. This phenomenon is at present inexplicable.

The percentage rate of growth of the uterus is greater than that of the ovary save at the height of puberty, or at the period immediately succeeding the first appearance of corpora lutea. This shift is exhibited in both series.

Combining this fact with the fact that the post-pubertal drop in percentage rate of growth of the ovary precedes and is greater than that of the uterus, it is clear that the development of the uterus lags behind that of the ovary, and that it is not completed during puberty when complete functional effectiveness of the ovary is attained.

As pointed out earlier (28) these data lead to the conclusion that the production of better animal stock from females mated some time after the attainment of sexual maturity as compared with stock resulting from pubertal or immediately post-pubertal matings, is a consequence of the more mature state of development of the uterus, since it is clear that an incompletely developed uterus is incompletely equipped to provide optimum nutritional conditions for the fertilized ovum.

If the ovary-uterus differential development holds in man, the sociologi-

cal implications of these observations are far reaching. They indicate that the physical inferiority of those races which practise child marriage

TABLE 3

The absolute and the relative percentage increments of the testes, ovaries, epididymis and uterus

AGE SERIES	THYROPARATHYROIDECTOMIZED			PARATHYROIDECTOMIZED		
	Controls	Tests	T/C	Controls	Tests	T/C
			Testes			
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
23	1,747.57	1,625.23	93.0	1,704.01	1,448.89	85.0
30	705.00	635.69	90.2	735.11	679.10	92.4
50	135.74	117.45	86.5	133.08	132.94	99.9
65	77.75	67.66	87.0	79.35	55.33	69.7
75	39.87	23.88	59.9	38.94	11.42	29.3
100	19.44	8.00	41.2	19.44	16.87	86.8
			Ovaries			
23	536.48	337.18	62.9	494.51	393.21	79.5
30	429.94	301.82	70.2	457.65	367.66	80.3
50	209.00	73.55	35.2	178.57	123.34	69.1
65	83.43	42.28	50.7	80.94	43.89	54.2
75	44.66	−1.65	−3.7	37.22	6.18	16.6
100	11.42	−26.61	−233.0	11.42	−10.73	−94.0
			Epididymis			
23	3,859.91	3,345.63	86.7	3,920.49	3,009.55	76.8
30	2,343.11	1,927.38	82.3	2,412.10	2,127.83	88.2
50	705.52	611.52	86.7	708.23	648.85	91.6
65	335.24	270.32	80.6	357.13	266.01	74.5
75	194.85	147.58	75.7	201.34	108.88	54.1
100	65.67	37.39	56.9	65.67	50.97	77.6
			Uterus			
23	3,215.82	2,453.55	76.3	3,272.39	2,686.34	82.1
30	3,490.41	2,437.24	69.8	3,460.40	2,484.93	71.8
50	481.68	232.32	48.2	385.49	283.54	73.6
65	150.62	93.93	62.4	145.69	99.84	68.5
75	126.89	37.71	29.7	98.10	34.19	34.9
100	35.03	−35.58	−101.6	35.03	−10.92	−31.2

is in part due to the relative retardation of uterine development. They are evidence against child-bearing before full physical development has been reached.

With this analysis of the control animal, we can turn to the effects of thyroid and parathyroid deficiency.

The mean weights of the testis, ovary, epididymis and uterus of the various groups together with their probable errors are given in table 2. The adjusted values were computed by the method given elsewhere (3).

As usual the analysis of the effect of the glandular deficiencies on growth is made from the values for the percentage rate of growth of the tests in terms of that of their controls. These are given in table 3 together with the absolute percentage increments as computed from the data in table 2. The chief point to be taken from these absolute percentage rates is that inter-organ differences in value in the control are not indices of inter-organ differences in growth retardation subsequent to thyroid or parathyroid removal. This has been discussed in earlier papers (3), (29).

For visualization the $\frac{\text{Test}}{\text{Control}}$ ratios of table 3 will be given in a series of charts, from which the inter-sex, inter-organ, and inter-age comparisons can readily be made. In these charts control growth is represented by the flat black columns (100 per cent), and test growth by the outline columns. The order of sequence is given by the superscript.

THE INFLUENCE OF THYROID DEFICIENCY. *Ovary growth.* On chart 1A the growth of the ovary is compared with that of the body in weight after thyroid removal at the stated ages. Ovary growth is considerably retarded by thyroid deficiency. The general course of change in degree with change in age at the time of glandular removal is the same in direction as that of the body as a whole. And up to the age when the pubertal adjustment is practically completed (75 days), the retardation is of the same order of magnitude as that of the body. Thereafter the reaction is greater.

The picture leads to the conclusion that the growth processes of the ovary are similar to those of the body as a whole in their relation to thyroid deficiency, and that their response is to be interpreted on the same basis (1), i.e., in terms of reduction of the metabolic level.

Consistent with this is the fact that the small though invalid degree of weight association between ovary and thyroid in the adult female (0.187 ± 0.071) is entirely dependent on the body size. This is shown by the drop in value to -0.030, when body weight is held constant by the method of partial correlation.

The observations of Hatai (30) and others show that ovarian growth is retarded along with body weight in conditions of nutritional deficiency, a state to which, as has been pointed out elsewhere (1), thyroid deficiency is essentially analogous.

Puberty introduces another factor. The incidence of the marked increase in growth retardation and deviation from the body weight response

(75 day old series) coincides with a marked decrease in the number of ova (15), which immediately follows the first appearance of corpora lutea.

The simultaneity of these phenomena indicates a causal relationship. I believe that the new factor of influence is the development of the incretory function of the ovary and its relation to that of the thyroid. The initiating agent is the ovary, a point of view which was stated in earlier papers (28), (31) and in which Lee (32) concurs.

A relatively increased sensitivity of the ovary to thyroid deficiency has been produced. Nevertheless this does not alter the fundamental similarity of its growth processes to those of the body as a whole.

It will be noted from chart 1A that the growth of the ovary is, with one exception, more sensitive than that of the body. This is due to the organo-specific type of metabolism (1). The accentuation of the difference when the specificity becomes full-blown is confirmation of the point of view. Now organic specificity determines the difference in degree of response of a given structure from that of the body as a whole or of its parts. It may also determine a difference in the character of the response with respect to the change in direction with age at the time of thyroid removal. If it does this, indication is had that there is a specific growth relation of the organ to thyroid deficiency which is distinct from that of the body as a whole. If, on the other hand, the general character of the change in response to thyroid removal at different ages is the same as that of the body weight, indication is had that there is no specific relation to thyroid deficiency. This distinction, of course, also applies when two or more organs are being compared.

In this sense it is evident that the ovary shows no specific type of growth relation to thyroid activity.

Uterus growth. On chart 1B the growth of the uterus is compared with that of the body after thyroid removal at each of the stated ages. The reaction is in general quite the same in type as that exhibited by the ovary.

The conclusion is that the growth processes of the uterus are similar to those of the body as a whole in their relation to thyroid deficiency, and that their response is to be interpreted on the same basis (1).

There is, however, some association between uterus and thyroid weight in the adult female which is independent of body weight. The coefficient of correlation between the two former is positive (0.367 ± 0.053) and is not greatly lowered when the latter is held constant by the method of partial correlation (the first order coefficient is 0.214). This relation may be a participant in the uterine regression of the 100 day old series.

Reports found in the literature (33) show that uterus growth is retarded or even rendered retrogressive by nutritional deficiencies. The uterus response to thyroid deficiency is thus consistent with the generalization noted earlier.

The generally lesser retardation of uterus growth as compared with body weight growth after thyroid removal is simply an expression of the relatively lesser degree of dependency of the growth processes of this organ on the maintenance of a normal metabolic level.

The conclusion is justified that the uterus shows no specific type of growth relation to thyroid activity.

Uterus growth vs. ovary growth. On comparing chart 1B with chart 1A it is seen that the uterus conforms more closely than does the ovary to the changes in growth retardation of the body weight with age at the time of thyroid removal. On chart 2A the growth of the uterus in the thyroidless rats is compared with that of the ovary. It is to be noted that although the change in degree of retardation of the former with change in age at time of thyroid removal is, in general, the same in direction as that of the latter, yet the uterus-ovary association is less close than the uterus-body weight association.

These relations indicate that the growth of the uterus, though similar to that of the ovary, is not necessarily specifically associated therewith as might be expected; but that the response of each is determined by the relation of the individual growth metabolism to the growth processes of the body as a whole. This distinction is supported by the probability that the greater sensitivity of the ovary is conditioned by an ovary-thyroid incretory relation.

It was shown in the introduction that the uterus is still growing at its developmental level rate during the growth period from 75 to 100 days of age, and that the ovary drops during this period from the developmental level to the level characteristic of the functionally mature organ. It is not until the growth period from 100 to 150 days that the uterus growth rate drops to the adult level (table 1). In view of these facts, it is to be expected that up to the time of the cessation of the developmental rate of growth the growth of the uterus as such or its response to thyroid deficiency, would be but little subject to the incretory activity of the ovary; but that with the drop to the level characteristic of the functionally mature organ, the uterus would come under the influence of the gonad, and that this would then, if at any time, be an effective participant in the growth reaction to thyroid lack. That such occurs at this time is seen from the fact that thyroid removal at 100 days of age (the time of the drop in growth rate to the adult level) is followed by a shift in the relative growth retardation of uterus and body weight.

From these facts it can be concluded that the ovary incretory influence participates in determining the degree of the growth reaction of the uterus to thyroid deficiency only when the full functional relationship between the two organs is established along with the attainment of the functional maturity of the latter. This participation does not alter the fundamental

relation of the growth processes of the uterus to those of the body as a whole.

Consistent with this is the fact that the ovary-uterus weight correlation in the adult rat is higher (0.427 ± 0.050), though not to a statistically valid degree, than the uterus-body weight association (0.367 ± 0.053), and the fact that a large part of the latter is conditioned by ovary influence since holding this constant by the method of partial correlation results in the lower first-order value of 0.178.

An earlier study has shown that the reproductive ability of the female albino rat is disturbed by thyroid deficiency (34). The disturbance was attributed to the lowering of the metabolic level and the consequent production of a state of partial inanition. It is seen from this study that a secondary contributing factor is the marked retardation of development of the ovary and uterus. The underlying cause as brought out in this and preceding analyses (1) is, however, the general metabolic disturbance. Confirmatory of this belief is the fact reported by Lee (32) that the oestrus cycle is lengthened after thyroid removal, and the fact that nutritional insufficiency produces similar reactions.

It may have been noted that exact age values are being used in the discussion. These are solely to be taken as reference points, for the beginning and end of these processes are at present incapable of exact determination.

Testis growth. On chart 10 the growth of the testis is compared with that of the body in weight subsequent to thyroid removal at the stated ages. It is seen that up to the time of practical completion of the pubertal adjustment (75 days of age) the growth of the testis is but slightly retarded by thyroid deficiency. Body weight growth is retarded to a much greater degree. Moreover, the degree of retardation of the testis during this period does not progressively increase with increase in age at time of thyroid removal as does that of the body. After this time, however, an increased retardation of testicular growth is exhibited and the degree progressively increases. The pubertal adjustment determines a similarity in growth response of testis to that of the body.

The conclusion is that up to the age of practical completion of the pubertal adjustment the growth of the testis is largely independent of thyroid deficiency and of body growth as related thereto. The growth processes of the testis up to puberty are, therefore, dissimilar from those of the body in general. After this they become more like those of the body and hence similarly subject to thyroid deficiency.

The pertinent correlation coefficients in the sexually mature rat are consistent with this conclusion. They show that the weight of the testis is only related to that of the thyroid through the influence of the factors represented by total body size, and not through any specific thyroid-testis

association. The coefficient of correlation between thyroid and testis is 0.218 ± 0.069. When the general size factors carried by the body weight have been eliminated by the method of partial correlation, the first order coefficient is -0.041. Combining this with the fact that the association between testis weight and body weight in the adult rat is high and positive (0.581 ± 0.041) it is clear that the change in type of response which occurs in the 75 day old series is due to an adjustment of the growth processes of the testis to a type more like those of the body as a whole. In this shift the dependence of the growth of the testis on the effectiveness of the growth processes of the body as a whole comes into expression. The significance of puberty in this shift deserves discussion.

From the introduction it is clear that the period from 23 days of age to puberty is a period of rapid growth of the testis: that at this time growth by increase in cell number is a large proportion of total growth: and that with the completion of this phase of development, the percentage rate of growth abruptly drops, and growth by increase in cell number becomes a lesser proportion of total growth. Contributive to the latter reaction is the release of the mature products of the cytogenic activity.

Now it was concluded from the evidence given in earlier papers (1), (2) that the progressive increase in growth retardation with increase in age at the time of initiation of thyroid deficiency, is essentially an expression of the diminution in the amount of growth attributable to cell division, and that growth by increase in cell number is less retarded by thyroid lack, than is growth by increase in cell mass. The deduction from this is that the thyroid is more concerned in the latter than in the former.

This being so it would be expected that an organ such as the testis, a large proportion of the total growth of which is, up to its functional maturity, a matter of increase in cell number, would show but little retardation subsequent to thyroid removal; but little if any progressive increase in retardation during the time when the organo-specific type of growth is at its height, and but little if any concordance with the age difference in response of the body as a whole in which growth by increase in cell number is obviously a lesser proportion of total growth. However, when this specific phase of development is completed, with the attainment of the pubertal adjustment, and when the products of the testicular reproductive functional activity have an outlet,—then it is to be expected that the growth response of the testis to thyroid deficiency would (unless there is a specific testis-thyroid relationship) tend to approximate that of the body as a whole. The agreement between assumption and fact is obvious.

The resistance of the testis may possibly be factored by the increase in cholesterol and phosphatides of the blood, a reaction which Baumann and Holly (36) and others report as occurring in conditions of thyroid deficiency.

The growth of the testis is less retarded than that of the body in weight. This is due to the probability that growth by increase in cell number is at these times a greater proportion of total growth in the former than in the latter.

In view of the foregoing there is no need to postulate a specific testis-thyroid growth relation.

Testis growth vs. ovary growth. The response of the ovary to thyroid deficiency differs from that of the testis in that it is similar to that of the body weight, while that of the testis pursues another course up to the time when the pubertal adjustment has practically been completed (chart 1A and C).

The objection might be raised that the explanation of the testicular response is wrong, since it is to be *a priori* presumed that a large proportion of the total growth of the ovary up to functional maturity is likewise due to growth by increase in cell number. This objection is lessened by the fact, shown by Arai (15), that the total number of ova in both ovaries does not increase during the growth period from 26 to 64 days of age, but remains approximately constant at a figure of 10,000. Between the ages of 64 and 70 days the number abruptly drops to 6600, at which level it remains for the rest of the period of observation used in these studies. Such a picture is not consistent with the idea of a large growth by increase in cell number.

The basis of the difference, then, lies in the respective difference of testis and ovary growth with respect to the proportion of total growth represented by growth by increase in cell number.

The relative growth response of the ovary and testis to thyroid removal is seen on chart 3A. It is evident that the ovary is more retarded by thyroid deficiency than is the testis.

It is seen from table 3 that the normal percentage rate of growth of the reproductive system of the male is greater than that of the female and that the resistance to the effects of thyroid deficiency is also greater. The two are, however, not correlatable, as a reference to the earlier discussions will show. The sex difference is not due entirely to the general body difference in the sense of the part following the whole. Nor is it due to any rough functional analogy. Rather is it due to the fact that a relatively larger proportion of the total growth in the testis is growth by increase in cell number, the which is more resistant to thyroid deficiency than is growth by increase in cell size.

The increase in the sex difference in gonad response to thyroid deficiency in the 75 day old series, the approximation of that of the testis to that of the body weight and the increase in degree of deviation of that of the ovary from that of the body weight, combine to produce the idea that there is a greater incretory association between ovary and thyroid than between testis and thyroid. Data from man point in the same direction. While

they are confirmatory, the data from the rats afford an explanation in that they indicate the greater incidence of thyroid disturbance in the female can be attributed to a sex-specific gonad-thyroid incretory relationship, which is not exhibited in the male to any significant degree, and that the initiating agent arises in the ovary.

Epididymis growth. On chart 1D the growth of the epididymis is compared with that of the body in weight subsequent to thyroid removal at the stated ages. The reaction is in general similar in type to that of the testis.

Turning now to chart 2C, where the growth of the testis in the thyroidless rats is compared with that of the epididymis, it is seen that the degree of retardation is essentially the same for both organs up to the time of practical completion of the pubertal adjustment. From here the degree of difference is noteworthy.

The conclusion is that up to the age of practical completion of the pubertal adjustment the growth processes of the epididymis are similar to those of the testis and dissimilar from those of the body as a whole in their reaction to thyroid deficiency. After this stage of development the growth processes of the epididymis become like those of the body and hence similarly subject to thyroid deficiency.

Since no data are available on the growth type of the epididymis, an interpretation by the same principle as sufficed for the testis is unjustified.

The pertinent coefficients of correlation substantiate the selective associations observed. The degree of weight association between testis and epididymis in the adult male is higher (0.839 ± 0.018) than that between the body weight and either testis (0.612 ± 0.038) or epididymis (0.639 ± 0.036). These latter values are strongly conditioned by the testis-epididymis association, since stabilization for epididymis in the first case, and for testis in the second, by the method of partial correlation brings the values to 0.181 and 0.293 respectively. Finally the testis-epididymis association is but weakly influenced by the general factors for organ size carried by the body weight, for when this is held constant the partial correlation coefficient is 0.737 as against the unstabilized value of 0.839.

This demonstrates a high degree of specific weight association between testis and epididymis independent of body weight. The basis of the relation probably lies in the nature of the structural and functional association of the two.

It is this normal interrelationship which determines the similarity in response to thyroid deficiency. It is like that exhibited by the brain and spinal cord of similar structural and functional interrelation and is noteworthy because many other pairs of organs show no such specificity (36).

It was noted earlier that the pubertal increase in retardation is less in degree in the epididymis than in the testis. This is due to the fact that

at this time there is added to the normal growth of the organ an increase in weight due to the storage of spermatozoa. This interpretation is consistent with the facts brought out in the introduction.

The shift from an independent to a type of response like that of the body as a whole which occurs at puberty is consistent, as in the case of the testis, with the fact that the weight association between epididymis and thyroid in the sexually mature male, is practically entirely a matter of body size influence. This is shown by the fact that the epididymis-thyroid correlation which is validly positive when body weight factors are present (0.283 ± 0.067) drops to 0.014 when these are held constant.

The data show that there is no specific epididymis-thyroid growth relation.

Attention is called to the fact that the testis and epididymis, like the brain and spinal cord, show a high specific type of growth resistance to nutritional (34) as well as thyroid deficiencies. The cumulative data are support for the belief that the growth retardation of thyroid lack is an expression of a condition of essential undernutrition in the sense developed in the earlier analysis (1).

Epididymis growth vs. uterus growth. Although the growth reaction of the uterus is greater than that of the epididymis to thyroid removal (chart 3B), the two are quite similar in the matter of their concordance with that of their respective gonads.

The degree of difference between uterus and epididymis (chart 3B) is less than that between ovary and testis. This indicates that the growth processes of the former are less dependent than those of the latter on the metabolic level.

THE INFLUENCE OF PARATHYROID DEFICIENCY. In an earlier paper the thesis was developed that the growth response to parathyroid deficiency is due to a lowering of the nutritional level with a resultant state of essential undernutrition (1). In this sense the growth reaction to parathyroid deficiency has a similar fundamental basis as that which follows thyroid removal. The inter-organ and inter-sex differences are thus interpretable on the same general principle.

Ovary growth. On chart 4A the growth of the ovary is compared with that of the body in weight after parathyroid removal at the stated ages. It is seen that the course of change in retardation degree with change in age at time of glandular removal is the same as that of the body: and that up to the age when the pubertal adjustment is practically completed (75 days), the retardation is of the same order of magnitude. Thereafter the response is greater.

The conclusion is that the growth processes of the ovary are like those of the body as a whole in their reaction to the disturbances produced by parathyroid removal. They are therefore to be interpreted on the same basis, i.e., in terms of a lowered nutritional level (1).

This is consistent with the reports on the growth response of the ovary to inadequate diets (33).

The simultaneity of the shift in relation between body weight and ovary and the change in state of the ovaries noted earlier shows that the degree of response of the ovary is specifically conditioned by its incretory activity. It is hard to decide if this is an expression of an ovary-parathyroid incretory relationship. The reaction may be merely an expression of the shift from the developmental to the adult type of growth (table 1). Such a conception is incomplete in the light of the findings published elsewhere (1), (2), (3), (4). These show that the pubertal surge in gonadal incretory activity plays a prominent part in the determination of normal growth, as well as in the growth response to thyroid and parathyroid deficiency. Such being the case, and in view of the reports in the literature that the parathyroidectomized organism is more prone to tetany during periods of alteration of ovarian incretory activity, it is possible that an incretory relationship exists between ovary and parathyroid, though not necessarily along a direct path. The activating agent probably arises in the ovary.

The ovary shows no specific type of growth relation to parathyroid activity.

Uterus growth. From chart 4B it is seen that uterus growth is retarded by parathyroid deficiency in a fashion quite like that of the body as a whole, but to a lesser degree.

This latter is an expression of the relatively lesser degree of dependence of the growth processes of the organ on the maintenance of the usual nutritional level.

Chart 1. Comparison of the growth of the ovary, A, uterus, B, testis, C, and epididymis, D, with that of the body in weight of the thyro-parathyroidectomized groups. Order of sequence given by the superscript.

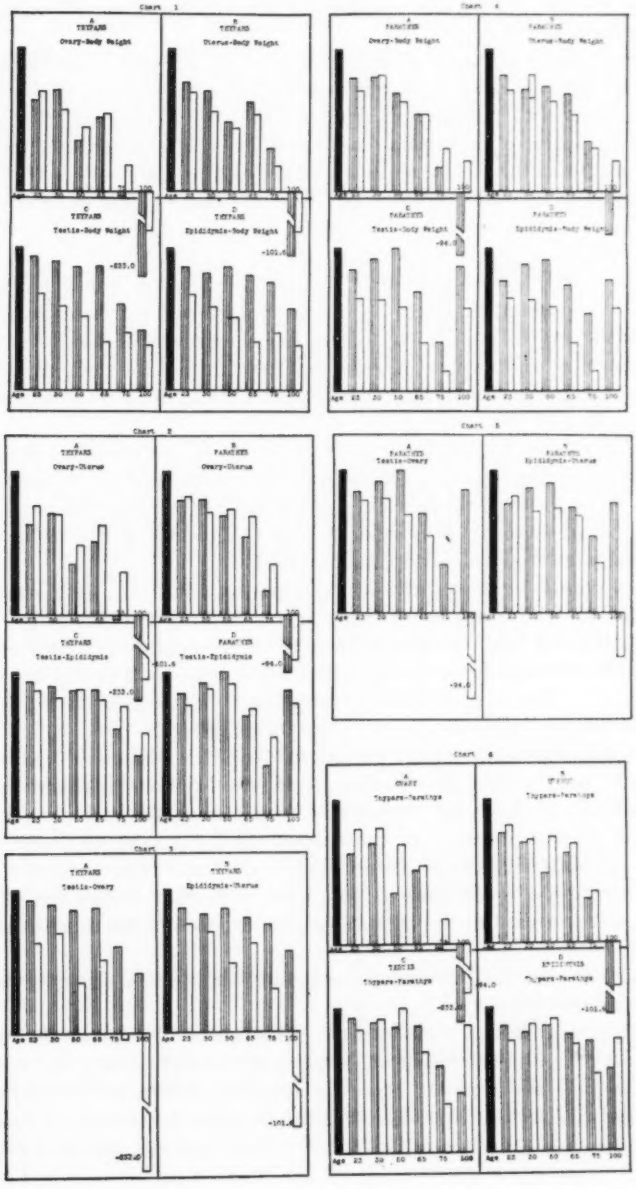
Chart 2. A, comparison of the growth of the ovary and uterus in the thyro-parathyroidectomized groups; B, of the ovary and uterus in the parathyroidectomized groups; C, of the testis and epididymis in the thyro-parathyroidectomized groups, and D, of the testis and epididymis in the parathyroidectomized groups. Order of sequence given in the superscript.

Chart 3. A, the growth of the testis compared with that of the ovary after thyro-parathyroidectomy. B, the growth of the epididymis compared with that of the uterus after thyro-parathyroidectomy. Order of sequence given in the superscript.

Chart 4. Comparison of the growth of the ovary, A, uterus, B, testis, C, epididymis, D, with that of the body in weight of the parathyroidectomized groups. Order of sequence given in the superscript.

Chart 5. A, the growth of the testis after parathyroidectomy compared with that of the ovary. B, the growth of the epididymis after parathyroidectomy compared with that of the uterus.

Chart 6. Comparison of the growth of the ovary, A, uterus, B, testis, C, and epididymis, D, in the thyro-parathyroidectomized with that in the parathyroidectomized groups. Order of sequence given in the superscript.



It is evident that the uterus shows no specific type of growth relation to parathyroid activity.

Uterus growth vs. ovary growth. From chart 2B and a comparison of chart 4B with chart 4A it is seen that the two organs react, in general, in similar fashion to parathyroid removal: that up to puberty (65 day old series) the degree of retardation is of the same order of magnitude for each, and that thereafter the uterus is less retarded than the ovary. It is probable that the incretory activity of the ovary participates in determining the response of the uterus to parathyroid deficiency during the period when full functional maturity is reached (100 day old series).

Testis growth. From chart 4C it is seen that up to the time when puberty exerts its characteristic effect on the general growth reaction to parathyroid deficiency (65 and 75 day old series) the growth of the testis is but little retarded, and its change is distinct from that of the body as a whole. The pubertal adjustment determines a similarity in growth response of the testis to that of the body.

The conclusion is that the growth processes of the testis are dissimilar from those of the body as a whole in their relation to the metabolic upset arising from parathyroid deficiency, up to the time of appearance of effective pubertal factors. It is evident that the growth of the testis is not specifically related to parathyroid activity.

Testis growth vs. ovary growth. Comparing chart 4C with chart 4A it is seen that the response of the ovary to parathyroid deficiency differs from that of the testis in its relation to the body weight reaction, and that the growth of the testis is consistently less retarded than that of the ovary (chart 5A). The basis of the interpretation is the same as that given for the like relation exhibited in the thyroidless groups.

Epididymis growth. Chart 4D shows that the relation of epididymis growth to body weight growth after parathyroid removal is essentially the same as that of the testis (chart 4C). Such being the case it is evident that the growth processes of the epididymis are similar to those of the testis. A pubertal shift in relation is also evident. The generally lesser resistance of the epididymis as compared with that of the testis (chart 2D) is an expression of the organo-specific response in the sense described earlier.

It is clear that the growth of the epididymis is not specifically related to parathyroid activity.

Epididymis growth vs. uterus growth. From chart 5B it is seen that uterus growth is generally retarded to a greater degree after parathyroid removal than is epididymis growth. Since the same sex difference is exhibited by the gonads (chart 5A) it is clear that the reproductive system of the male is more resistant than that of the female to this type of glandular deficiency.

THE RELATIVE INFLUENCE OF THYROID AND PARATHYROID DEFICIENCY. Turning from similarities to differences it is seen from chart 6A and B that the growth of the ovary and uterus is more retarded by thyroid than by parathyroid deficiency. From C and D on the same chart it is seen that the same relation exists in the case of the testis and epididymis of the 30, 50 and 100 day old series, and the opposite in the 23, 65 and 75 day old series. These relations are concordant with the differential response of the body weight. The difference is less in degree in the male. This is also like the body weight relations. Hence it must be concluded that these differences are largely determined by the dependence of the organs on the effectiveness of the growth processes of the body as a whole in their differential reaction to the incretory deficiencies.

The shift in relation between the degree of retardation caused by the two types of incretory deficiency which occurs only in the males of the 65 and 75 day old series, and the return in the 100 day old series to the relation exhibited in the pre-shift groups, shows that there is a sex-specific pubertal sensitivity to parathyroid deficiency in this sex. It cannot be said that a testis-parathyroid incretory relation is of specific influence on testicular growth. Hence the reaction must be attributed to the dependence of this organ on the general growth processes of the body, and their specific response to the combined influence of parathyroid deficiency and puberty.

SUMMARY AND CONCLUSIONS

A study is given of the normal course of development of the reproductive systems of male and female albino rats which serves as a basis of determination of the rôle of the thyroid apparatus therein.

The correlated data show that:

1. An improvement in dietary and environmental conditions brings about an earlier sexual maturity.

2. The reproductive system of the male reaches maturity at an earlier age than does that of the female.

3. The development of the uterus lags behind that of the ovary.

The sociological implications of these findings in their relation to man are briefly indicated.

The chief facts of interest in connection with the rôle of the thyroid apparatus in the growth of the reproductive system are as follows:

1. There is no apparent specific relation between the growth of the reproductive system of either sex and thyroid or parathyroid activity. The growth retardation which occurs is attributable to the general metabolic disturbance which results in a condition of essential undernutrition, and the dependence of the parts of the body on the effectiveness of the growth processes of the whole.

2. The ovary and the uterus follow the body weight in its changes in growth retardation after glandular removal at the stated ages. The degree of retardation is of the same order of magnitude until the practical completion of the pubertal adjustment, when the surge in ovarian incretory activity determines a relatively increased sensitivity of the reproductive system. This is evidence for an ovary-thyroid and ovary-parathyroid incretory relationship, the basis of which lies in the ovary.

3. Evidence is had that the incretory activity of the ovary conditions the response of the uterus to the glandular deficiencies, when and only when, the latter has reached the period in which it attains its full functional maturity.

4. The testis and epididymis are less sensitive to thyroid and parathyroid deficiency than is the body as a whole. Prior to the pubertal adjustment they do not follow the body weight change with change in age at time of glandular removal. After the readjustment they show a direction of change like that of the body. The difference is attributable to a difference in the relative proportion of total growth represented by growth by increase in cell number. The pubertal adjustment decreases this difference and hence the growth reaction of the reproductive system approximates in kind that of the body as a whole.

5. The marked concordance of testis and epididymis response both in degree and kind is attributable to the close functional and structural association between the two.

6. The relations outlined are consistent with the pertinent coefficients of correlation.

7. Evidence is had that a factor in the greater incidence of thyroid disturbances in girls and women as compared with boys and men, is the presence of a greater incretory relation between ovary and thyroid, than is evident between testis and thyroid.

8. The relations of the growth responses of the various organs to those of the body as a whole and to each other, subsequent to glandular removal at the different ages, confirm the belief as to the rôle of the thyroid apparatus in growth developed in an earlier study (1).

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ADDITIONAL DATA ON THE SPECIFICITY OF LUCIFERIN AND LUCIFERASE, TOGETHER WITH A GENERAL SURVEY OF THIS REACTION

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Since the discovery of luciferin and luciferase by Dubois (1887), I have tested for the presence of these substances in a large number of forms and have also investigated the production of light when the luciferin and luciferase of *Cypridina hilgendorfii*, an ostracod, is mixed ("crossed") with luciferin and luciferase solutions of other animals (Harvey, 1922). The investigation has been continued from time to time, as material could be obtained, and the experiments with additional luminous forms, not previously investigated or in which the results were doubtful are herein reported.

By luciferin and luciferase solutions, I mean solutions so prepared that they should contain luciferin and luciferase, even though the luciferin-luciferase reaction cannot be demonstrated for the particular luminous organism in question. Detailed methods of preparation are given in my first paper. In general luciferin solution is prepared by quickly heating to 70° or to boiling a luminous extract of a luminous animal, or by pouring boiling sea water or fresh water on a luminous tissue, grinding in a mortar, and cooling. If luciferin and luciferase were present in the animal, the latter would be destroyed by the heat, leaving the luciferin in solution. Luciferase solutions are prepared by extracting luminous tissues with fresh water or sea water, often adding some cytolytic agent like saponin or chloroform to break up the cells and granules, and allowing the extract to stand till the luminescence disappears. Any luciferin will be completely oxidized leaving the luciferase behind. Both luciferin and luciferase solutions are dark but luminescence should result on mixing the two if the luciferin-luciferase reaction is positive.

The results are given in the accompanying table. The *Cypridina* luciferin always gave a brilliant luminescence with *Cypridina* luciferase. Other forms which I (1922) have previously tried, all of which gave a negative luciferin-luciferase reaction and no luminescence when "crossed"

[1] Many experiments carried out while appointed to the A. A. A. S. Table at Naples.

TABLE 1

ORGANISM	"CROSS"					REACTION
Bacteria	Cypridina	luciferase	+	Bacterium	luciferin	Negative
	Cypridina	luciferin	+	Bacterium	luciferase	Negative
B. phosphorescens	Bacterium	luciferin	+	Bacterium	luciferase	Negative*
Fungi	Cypridina	luciferase	+	Panus	luciferin	Negative
	Cypridina	luciferin	+	Panus	luciferase	Negative
P. stipticus	Panus	luciferin	+	Panus	luciferase	Negative
Radiolaria	Cypridina	luciferase	+	Thalassicolla	luciferin	Negative
	Cypridina	luciferin	+	Thalassicolla	luciferase	Negative
	Thalassicolla	luciferin	+	Thalassicolla	luciferase	Negative
C. inerme	Cypridina	luciferase	+	Collozoum	luciferin	Negative
T. nucleata	Cypridina	luciferin	+	Collozoum	luciferase	Negative
	Collozoum	luciferin	+	Collozoum	luciferase	Negative
Ctenophores	Cypridina	luciferase	+	Mnemiopsis	luciferin	Negative
	Cypridina	luciferin	+	Mnemiopsis	luciferase	Negative
	Mnemiopsis	luciferin	+	Mnemiopsis	luciferase	Negative
	Cypridina	luciferase	+	Beroe	luciferin	Negative
	Cypridina	luciferin	+	Beroe	luciferase	Negative
M. Leidyi	Beroe	luciferin	+	Beroe	luciferase	Negative
B. ovata	Cypridina	luciferase	+	Eucharis	luciferin	Negative
E. multicornis	Cypridina	luciferin	+	Eucharis	luciferase	Negative
	Eucharis	luciferin	+	Eucharis	luciferase	Negative
Medusae	Cypridina	luciferase	+	Pelagia	luciferin	Negative
	Cypridina	luciferin	+	Pelagia	luciferase	Negative
P. noctiluca	Pelagia	luciferin	+	Pelagia	luciferase	Negative
Annelids	Cypridina	luciferase	+	Tomopteris	luciferin	Negative
	Cypridina	luciferin	+	Tomopteris	luciferase	Negative
	Tomopteris	luciferin	+	Tomopteris	luciferase	Negative
	Cypridina	luciferase	+	Acholoe	luciferin	Negative
	Cypridina	luciferin	+	Acholoe	luciferase	Negative
	Acholoe	luciferin	+	Acholoe	luciferase	Negative
T. helgolandica	Cypridina	luciferase	+	Microscolex	luciferin	Negative
A. astericola	Cypridina	luciferin	+	Microscolex	luciferase	Negative
M. phosphorea	Microscolex	luciferin	+	Microscolex	luciferase	Negative
P. caliendrium	Cypridina	luciferase	+	Polycirrus	luciferin	Negative
	Cypridina	luciferin	+	Polycirrus	luciferase	Negative
	Polycirrus	luciferin	+	Polycirrus	luciferase	Negative
Crustacea	Cypridina	luciferase	+	Copepods	luciferin	Negative
	Cypridina	luciferin	+	Copepods	luciferase	Negative
	Copepods	luciferin	+	Copepods	luciferase	Negative
Cypripina (?) sp.	Cypridina	luciferase	+	Cypripina (?)	luciferin	Positive
Copepods	Cypridina	luciferin	+	Cypripina (?)	luciferase	Positive
	Cypripina (?)	luciferin	+	Cypripina (?)	luciferase	Positive
Ophiurians	Cypridina	luciferase	+	Amphiura	luciferin	Negative
	Cypridina	luciferin	+	Amphiura	luciferase	Negative
A. squamata	Amphiura	luciferin	+	Amphiura	luciferase	Negative
Molluscs	Cypridina	luciferase	+	Heteroteuthis	luciferin	Negative
	Cypridina	luciferin	+	Heteroteuthis	luciferase	Negative
	Heteroteuthis	luciferin	+	Heteroteuthis	luciferase	Negative
H. dispar	Cypridina	luciferase	+	Pholas	luciferin	Negative
P. dactylus	Cypridina	luciferin	+	Pholas	luciferase	Negative
	Pholas	luciferin	+	Pholas	luciferase	Positive
Balanoglossids	Cypridina	luciferase	+	Ptychodera	luciferin	Negative
	Cypridina	luciferin	+	Ptychodera	luciferase	Negative
	Ptychodera	luciferin	+	Ptychodera	luciferase	Negative
P. sp. (?)	Cypridina	luciferase	+	Balanoglossus	luciferin	Negative
B. minutus	Cypridina	luciferin	+	Balanoglossus	luciferase	Negative
	Balanoglossus	luciferin	+	Balanoglossus	luciferase	Negative

* Gerretsen (1920) has reported positive results with *Photobacterium javanense* luciferin and luciferase but negative with *Photobacterium phosphorescens*.

with *Cypridina* luciferin and luciferase are: the cystoflagellate, *Noctiluca miliaris;* the medusae, *Aequorea forskala* and *Microcoma cellularia;* the pennatulids, *Pennatula phosphorea* (?) and *Cavernularia Haberi* from Japan, and *Ptylosarcus* sp. (?) from Friday Harbor, Washington; the ctenophora, *Bolina* sp., (?); the Annelids, *Harmithoë imbricata* and *Chaetopterus variopedatus;* the crustacean, *Meganyctiphanes norvegica,* the balanoglossid, *Ptychodera* sp. (?) from Bermuda; the ascidian, *Pyrosoma* sp. (?) from Monaco, and the fish *Photoblepharon palpebratus*[2] and *Anomalops katoptron*,[2] from the Banda Islands.

Fire-flies give the luciferin-luciferase reaction but do not (at least not *Luciola viticollis*) react with *Cypridina* and the same is true of *Odontosyllis phosphorea.* The elaterid beetle, *Pyrophorus noctilucus,* gives the luciferin-luciferase reaction (Dubois, 1885) but I have never tried the cross with *Cypridina.*

The sponge, *Grantia* sp. from Friday Harbor, Washington (Harvey, 1921); the squid, *Watesenia scintillans* from Japan; the myriapod, *Geophilus* sp. from Java; and the fish, *Monocentris japonica* from Japan, give negative luciferin-luciferase reactions. I have never tried the "cross" with *Cypridina.*

Thus it will be observed that only ostracods, fire-flies (including *Pyrophorus*) *Pholas dactylus* and *Odontosyllis* among the 40 genera, representing some 23 different groups, which I have personally investigated, give the luciferin-luciferase reaction. Hickling (1925) has recently reported a positive reaction under certain conditions in the fish, *Malacocephalus levis.*

As *Cypridina* luciferase will give luminescence on mixing with *Cypridina* luciferin in a concentration of one part to a billion of water and vice versa (Harvey, 1923) it would seem that luminescence should appear with crosses of *Cypridina* and other luminous forms, provided the foreign extract contained even a trace of luciferin (or luciferase, respectively). As no light appears, these results again confirm the specificity of the reaction. Only where we mix extracts of two genera of ostracods do we get reciprocal luminescence.

Perhaps the specificity of the reaction is not so unusual, but we must return to the question as to why the luciferin-luciferase reaction is not demonstrable in many luminous animals, in fact in the majority of forms. I have as yet no certain explanation of this fact, for it would seem that in organisms with abundant luminous slime, like *Polycirrus* or *Pelagia* we should be able to demonstrate a luciferin-luciferase reaction. In *Heteroteuthis* also there is abundant pure luminous material and in copepods, so closely allied to ostracods, the luminescence is similar to *Cypridina,* yet the luciferin-luciferase reaction is negative.

[2] Luminescence due to luminous bacteria. Harvey, 1922b.

My experience has been so great that I am loath to attribute negative results to faulty technique. However, there is always the possibility that in some forms luciferin is very easily oxidized without light production and is so oxidized during its preparation.

Accordingly I have prepared luciferin not only in absence of oxygen but under conditions where any oxyluciferin previously formed should be reduced to luciferin again. The luminous tissue is placed in a test-tube with some platinized asbestos and pure hydrogen[3] bubbled through for 15 to 30 minutes. That this is ample time to remove any traces of oxygen and to reduce oxyluciferin is shown by the absence of luminescence, as well as by other experiments using *Cypridina* luciferin. The material is then heated in the reducing atmosphere to 70°C. or to boiling and cooled. We should certainly expect luciferin prepared in this way to luminesce with luciferase yet I have never observed any light although many forms have been tested. These include luminous bacteria, *Balanoglossus minutus*, *Amphiura squamata*, *Chaetopterus variopedatus*, *Microscolex phosphoreus*, Copepods, *Panus stipticus*, *Acholoë astericola*, *Pennatula phosphorea*, *Mnemiopsis leidyi* and *Beroë ovata*. I have found[4] that the oxygen cannot be removed from extracts of the medusa, *Pelagia noctiluca*, or the ctenophores *Beroë ovata* and *Eucharis mullicornis* by the above method, and it is probable that *Mnemiopsis* behaves as the other ctenophores, although I have not actually tried it. However all the other mentioned forms require oxygen. Since we obtain negative results in a hydrogen atmosphere I feel convinced that rapid oxidation of luciferin is not the reason for the failure of the luciferin-luciferase reaction.

We are therefore forced to fall back on the explanation I suggested in 1922, that in these forms luciferase is not a catalyst but that there is just enough luciferase present to be completely utilized by the luciferin. Consequently, during the preparation of a "luciferase" solution, all the luciferase is used up. An analogous case is presented in the oxidase of potato which combines with definite amounts of chromogen to give a black pigment, and will not oxidize indefinite amounts of chromogen.

It is practically universal to find the luminous material of animals in the form of granules. Perhaps we may regard the granules in the forms which give no luciferin-luciferase reaction as containing just the right amount of luciferin and luciferase mixed together for luminescence. In these forms luminescence seems to occur on dissolution (granulolysis) of the granule, a phenomenon especially marked in coelenterates. In *Cypridina* the luminescence is not dependent on visible granules but occurs

[3] Made by passing hydrogen over heated platinized asbestos in a quartz tube and conducting through a system having only glass or lead connection, since oxygen diffuses through rubber tubing.

[4] Not yet published.

in granule-free solutions, although the luminous gland cells of *Cypridina* contain at least two kinds of granules which form luciferin and luciferase on dissolution.

Of all the new forms investigated I should have thought the copepods most likely to give the luciferin-luciferase reaction, in view of their close relation to *Cypridina* and similarity in luminous gland cells (Giesbrecht, 1895). Yet the tests are very clearly negative. There is the possibility that copepod luciferase is digested by ferments of the alimentary canal since the whole animals are used in preparing a "luciferase" solution. However, whole animals are used in preparing *Cypridina* luciferase which does give the reaction, while only the luminous tissue in many other forms which show no luciferin-luciferase reaction.

My attempts to obtain luciferin from an extract of copepods which should contain oxyluciferin, the oxidation product of luciferin, by reduction with hydrogen and palladinized asbestos have also failed. This experiment is easily carried out by extracting copepods with water, allowing all the luminescence to disappear, when all luciferin should oxidize to oxyluciferin, then adding Pt asbestos and passing pure hydrogen through the solution for 30 to 60 minutes. If any reduction of oxyluciferin has occurred, the extract should luminesce on shaking with air. No luminescence occurs.

The same experiment has been tried both with the slime of *Pelagia* (treated with chloroform to cause luminescence and consequent oxidation of luciferin) and also with *Chaetopterus*, but with negative results. The oxyluciferin of *Cypridina* is very easy to reduce by this method, as also by means of sodium hydrosulphite.

Dubois (1919) believes that reduction phenomena play a part in the luminescence of *Pholas*, and my experiments in reducing the oxyluciferin of *Pholas dactylus* have convinced me that this can be done with Pt asbestos and hydrogen but the amount of luciferin obtained is small and not nearly so striking as in the case of *Cypridina*. Attempts to reduce *Pholas* oxyluciferin with sodium hydrosulphite have failed.

Of all the luminous forms which I have studied, ostracods are unique in the delicacy of the reaction. The greatest care must be taken in washing test-tubes that have contained *Cypridina* luciferin or luciferase to make sure that the last traces are removed before they are used for some other material. The luminescence is far brighter than can be obtained in the case of the fire-fly *Pholas* or *Odontosyllis*, and is also far less complicated by the phenomenon of granulolysis or cytolysis, which I intend to discuss in another paper. Indeed my experience has brought me to the conclusion that Cypridina is rather unique among luminous organisms and far superior for study to any form I am acquainted with, not excepting *Pholas*.

The experiments herein reported have been carried out at various places

where the material could be obtained over a period of some years. At Naples during the winter of 1925–1926 I obtained a large number of luminous animals including *Pholas dactylus* (sent to Naples from Taranto), *Balanoglossus minutus*, *Amphiura squamata*, *Thalassicolla nucleata*, *Colozoun inerme*, *Pennatula phosphorea*, *Beroë forskalii* and *ovata*, *Eucharis multicornis*, *Pelagia noctiluca*, *Microscolex phosphorea*, *Acholoë astericola*, copepods, and *Sepiola*.[5] During a short visit to Messina, Sicily, I was able to obtain many copepods, deep-sea fish, and the remarkable squid, *Heteroteuthis dispar*. I take great pleasure in thanking Doctor Reinhardt Dohrn, Director of the Naples Station, and Doctor Luigi Sanzo, Director of the Istituto Centrale di Biologia Marina, Messina, with their respective staffs, for many courtesies during my visit.

The bacteria were isolated by Mr. T. F. Morrison from fish and appeared to be *bacterium phosphorescens*, Fischer. *Mnemiopsis leidyi* was obtained at Woods Hole in August, 1924 and *Panus stipticus* in August, 1925.

The *Cypripina* was obtained at Montego Bay, Jamaica, in December, 1923, and the *Ptychodera* in Bermuda in December, 1921.

The experiments with *Tomopteris helgolandica*, *Polycirrus caliendrum*, and *Pholas dactylus* (in part) were carried out at the Laboratory of the Marine Biological Association at Plymouth, England. It gives me great pleasure to acknowledge the kindnesses of the Director, Doctor E. J. Allen, and the laboratory staff, during my occupancy of a table at the Station in August, 1923.

CONCLUSIONS

Among forty-one different genera of luminous animals, representing some twenty groups, personally investigated, only *Pholas dactylus*, ostracods, fire-flies (including *Pyrophorus*) and *Odontosyllis* give the luciferin-luciferase reaction. Hickling reports the fish, *Malacocephalus laevis*, to give it. The reaction is the exception rather than the rule.

Cypridina luciferin (or luciferase) will react with the luciferase (or luciferin) of two other genera of ostracods with luminescence, but with none of the other luminous animals, thirty-five genera having been tested, excluding *Malacocephalus*. The reaction is therefore highly specific.

In many cases the absence of the luciferin-luciferase reaction cannot be due to lack of luminous material or to destruction of luciferase by digestive ferments, or to spontaneous oxidation of luciferin. It is suggested that in these forms just enough luciferase is present to be completely used up by the luciferin. Possibly the two are combined in one granule whose luciferase becomes exhausted on luminescence.

No luminescence can be again obtained in extracts of copepods, *Pelagia*

[5] The luminescence of Sepiola is due to luminous bacteria.

or *Chaetopterus*, whose luminescence has disappeared, by reduction with Pt asbestos and hydrogen. This can be accomplished easily with *Cypridina* and less readily with *Pholas*. *Odontosyllis*, fire-flies, and *Malacocephalus* have never been tested.

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BIOLUMINESCENCE AND FLUORESCENCE IN THE LIVING WORLD

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From the Stazione Zoölogica, Naples

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Since the development by Wood (1903) and Lehman (1910) of absorption screens to absorb the visible but allow ultra violet light to pass, many observers (Stubel, 1911; von Prowazek, 1913; Hess, 1911; Heller, 1915) have described the fluorescence of tissues. In such a study the microscope can also be used (Lloyd, 1923; Lehman, 1913; Reichert, 1911; Heimstadt, 1911). Perhaps the fluorescence of the lens of the eye is best known, but most skeletal and supporting tissues such as bone, teeth, cartilage, nails, skin, tendon, etc., are markedly fluorescent and ordinary protein rich cells less so. The effect is easily observed on examining the objects in a dark room by the light from a small carbon arc (enclosed in a dark house and the light focused to a parallel beam) that has passed through a saturated solution of $CuSO_4$ and then through some of the ultra-violet glasses now in the market. The Corning G586A or Wood's ultraviolet glass is excellent for the purpose. Such an absorptive combination allows only a little red and blue light to pass, giving a very dim purple color, but much ultra-violet of wave-lengths near the blue end of the visible spectrum. This is what is meant by ultra-violet light when referred to in this paper, unless other wave-lengths are specially mentioned. Quinine, many dye solutions, anthracene, and some minerals are brilliantly fluorescent in this light as well as many extracts of plant and animal tissues. I have examined a large number of flowers of various colors for fluorescence but the results have been negative and rather disappointing. Orange blossoms are most fluorescent. Pollen is often brilliantly bluish fluorescent, as well as resinous parts.

The wax of the woolly aphid of alders (*Schizoneura tesselata*) is brightly bluish fluorescent. Cockayne (1924) reports fluorescent pigments in the wings of many lepidoptera. The annelid, *Chaetopterus*, and certain squid contain an orange fluorescent material in the liver. The unpigmented skeletal parts of invertebrates are fluorescent as in the vertebrates.

The fluorescence of these tissues and substances is an actual light emission through conversion of certain wave-lengths of the exciting ultra-violet

[1] Appointed to the A. A. A. S. table at Naples, October, 1925 to January, 1926.

light, which are absorbed, into the fluorescent wave-lengths emitted. Such light is not polarized and can be distinguished in this way from reflected light and the Tyndall phenomenon. We must believe that molecules are excited by the absorbed light and emit light of other wave-lengths, giving the fluorescent spectrum. It would seem quite possible that the photogenic material of luminous animals or chemiluminescent substances should be excited to luminesce by light of some wave-length, possibly ultra-violet. The first case in which attention was called to fluorescence and chemiluminescence was discovered by Kautsky and Zocher (1922) in unsaturated silicon compounds. These compounds are chemiluminescent on oxidation and fluorescent with the same colored light when exposed to ultraviolet radiation. These authors draw the conclusion that if a compound is fluorescent, the chances are that it may be made to luminesce by chemical means, and vice versa. Dufford, Calvert and Nightingale (1925) find some chemiluminescent Grignard compounds to be fluorescent, but their oxidation products are almost universally fluorescent. However, the fluorescent spectrum is not always the same as the chemiluminescent spectrum.

I have never observed any fluorescence of the brightly luminous material of *Cypridina* or its oxidation product, despite many attempts to demonstrate such a fluorescence, but in studying the inhibition of ctenophore luminescence in ultra-violet light, I have observed that ultra-violet fluorescence occurs after the luminescence has ceased. That is, after stimulation to luminescence and the luminescence having subsided, so that the animal was perfectly dark, the previously luminescent regions (radial canals in *Mnemiopsis*) would be markedly fluorescent in ultra-violet light. This effect gradually disappeared and seemed to be due to fluorescence of the oxidation product of the luminous material (Harvey, 1925).

Since then I have examined a considerable number of luminous forms and found several that give a beautiful fluorescence of the luminous organ. The easiest of these to observe is the fire-fly, whose organ is always fluorescent whether it has previously been very active or not. But I presume many luminous organs always contain some of the oxidation product of luminous material.

In examining luminous animals for luminescence it must always be remembered that protein is fluorescent so that the only thing of significance is a marked fluorescence in the luminous organ. I have noted also that the color is the same as that of the luminescence, which certainly adds further significance to the phenomenon. The luminous organs of dried fire-flies, as well as the elaterid bettle, *Pyrophorus*,[2] even specimens collected ten

[2] The color of the fluorescence of dried *Pyrophorus* luminous organ is more bluish than that of the fresh organ. The eyes of *Pyrophorus* and the fire-fly are also bluish fluorescent.

years ago, possess luminous organs which are brightly fluorescent. It is interesting to note that the fluorescence of dried *Photinus pyralis* is decidedly orange as compared with that of *Photuris pennsylvanica*, which is more greenish, just as the luminescence of the living animal. Fresh *Pyrophorus* luminous organs heated to boiling are also brightly fluorescent in ultra-violet light although all possibility of luminescence is gone. A perfectly dark Pyrophorus presents a beautiful appearance in ultra-violet light, the thoracic organs glowing exactly as if they were luminescent.

The investigated organisms which show no special fluorescence of luminous material are:

Luminous bacteria (particles of egg albumen are more fluorescent than a dense mass of the bacteria); fungi, *Panus stipticus;* the radiolarian, *Thalassicolla nucleata;* the anthozoa, *Pennatula phosphorea* and *Funiculina quadrangularis* (the skeleton of these forms is very fluorescent); the worms, *Thelepus cincinnatus* and *Tomopteris euchaeta;* the mollusc, *Pholas dactylus;* the squid, *Heteroteuthis dispar;* the ostracod, *Cypridina hilgendorfii; Balanoglossus minutus;* and the medusa, *Pelagia noctiluca.*

The luminous slime of the worm, *Chaetopterus variopedatus*, looks more fluorescent than ordinary slime but it is difficult to tell. The material in the liver appears of a beautiful golden color in ultra-violet light, as it streams from the organ into the sea water. The red liver of the squid, *Sepia elegans*, shows the same thing as did a colorless organ in *Loligo intermedia* that was probably liver or salivary gland, although its identity was not certain.

Tomopteris was observed under the microscope. The yellow luminous gland cells in each parapodium showed no fluorescence when ultra-violet light was reflected down on them by a mirror, even when the animal was strongly stimulated and its decidedly yellow luminescence had appeared.

The earthworm, *Microscolex phosphorea*, which produces a yellowish luminescent slime, observed in ultra-violet, was found to show yellowish florescent areas over the body. But as other species of non-luminescent earthworms showed the same effect I conclude that it is only fluorescence of the ordinary slime so co mon to those animals, which one observes.

One should certainly expect to find some fluorescence in *Pholas dactylus* where the luminous material is concentrated in the organs of Poli and in *Heteroteuthis* whose gland is densely crowded with brightly luminous photogenic granules, yet no fluorescence appears.

The organisms which do show a marked fluorescence are:

Beroë ovata. This ctenophore behaves just as does *Mnemiopsis*, except that the luminescence appears in a network of canals, branching from the radial canals instead of the radial canals alone. On stimulation this network is beautifully outlined in yellowish green luminescence and when the luminescence has subsided, the network is brightly fluorescent with

the same color in ultra-violet light. It is as if the animal were luminescent. Resting unstimulated animals, light-adapted[3] animals and dark-adapted animals which for some reason do not luminesce on stimulation show only the faint bluish fluorescence of protein in ultra-violet light. The fluorescent material disappears in 10 or 15 minutes and upon a second strong stimulation the phenomenon can be repeated again.

Ophiurians. Both *Amphiura squamata* and *Ophiopsila aranea* are brightly yellowish green fluorescent after they have been stimulated to luminesce strongly. The fluorescence is very marked and bright and can be observed under the microscope as a network of yellowish green fluorescent material on the plates. This fluorescence lasts for a long time and is very different from the bluish fluorescence of the skeletal parts themselves. In *Ophiopsila aranea* the fluorescence appears as bright points of yellow light evenly distributed over the plates. I have also noted the same yellow fluorescent particles on the feet, but do not know the meaning of this as Mangold (1910) describes the feet as non-luminous. Needless to say, the yellowish fluorescent light disappears when the ultra-violet is screened.

The worm, *Acholoë astericola*, shows the some phenomenon as the ophiurians. It is very easily stimulated to bright yellowish luminescence over the scales which cover the dorsal surface of the body. When examined in ultra-violet light, some worms are found to be brightly yellowish fluorescent over the scales, others not. But the latter, when stimulated to luminesce strongly, will also show the yellowish fluorescence in ultra-violet light after the luminescence has subsided. The material secreted from the gland cells described by Falger (1908) and Kutschera (1909) is evidently highly fluorescent and some of the worms, despite careful handling, have luminesced previously. The non-luminous, *Polynoë grubiana*, shows only the bluish fluorescence of skeletal parts when its scales are examined (both upper and lower sides) in ultra-violet light. The setae are brightly bluish-violet fluorescent.

Copepods. Giesbrecht (1895) found five species of copepods to be luminous at Naples.

In order to examine these animals for fluorescence it is necessary to use the microscope and a convenient method is to collect them in a fine mesh wire screen (bolting cloth will not do, as the silk is brightly fluorescent), place this on the stage of the microscope and direct ultra-violet light down on the animals from above by a mirror. One can then observe the greenish blue luminescence of the copepods from unicellular glands appearing here and there and that when this luminescence fades, many, although not all, of the originally luminescent spots are now brightly greenish blue

[3] By light-adapted animals I mean individuals which have been exposed to a bright light so that they no longer luminesce on stimulation.

fluorescent. In fact, it is only by screening off the ultra-violet light that one can tell the difference between bioluminescence and a fluorescence.

I should perhaps emphasize the fact that the use of ultra-violet light is a great aid in studying the exact location of luminescent regions in an animal with the microscope since there is usually sufficient general fluorescence of the organism to make its outline clearly visible without any glare in the eyes and bright fluorescent spots usually mean luminous organs. One can easily focus on these spots and then test the matter in complete darkness by stimulation.

During a short stay at the marine laboratory at Messina I had an opportunity to examine two species of deep sea fish, *Myctophum (Scopelus) Benoiti* and *Argyropelecus hemigymnus*, in a quite fresh condition, although no luminescence could be obtained, even on tearing them to pieces. The luminous organs of the first were brightly greenish fluorescent in ultraviolet light, while *Argyropelecus* showed no particular fluorescence. It is actually the photogenic cells and not the epidermal lens which is fluorescent in the case of *Myctophum*, as I determined by dissection.

A large number of luminous fish preserved in formalin and kindly presented to me by Doctor Luigi Sanzo, Director of the Messina Laboratory, were examined and many found to have luminous organs markedly fluorescent in ultra-violet light. These included *Myctophum Benoiti* and a number of other species, *Stomias boa*, *Chauliodus Sloani*, *Coccia ovata*, *Maurolicus Pennanti*, *Vinciguerria attenuata* and *Poveriae* and *Gonostoma denudatum*. The formalin preserved squid, *Histioteuthis bonelianus* had fluorescent luminous organs but not *Pyroteuthis margaritifera*.

It appears as if one might detect luminous organs by their fluorescence and even use preserved material for the purpose but caution must be exercised in laying too much stress on fluorescence as I observed a fresh scopelid fish, *Chloropthalamus Agassizi*, to have a beautiful yellowish-fluorescent crescent over the dorsal half of the eye-ball, yet Doctor Sanzo assures me that this form is not luminous. After two months in formol the fluorescent spot had disappeared. However, I do believe it is worth while to apply the fluorescence test to organs whose luminescent nature may be in dispute.

As we observe fluorescence of luminous organs that have been preserved in formol, or heated or dried, it is not surprising to find that the fluorescence appears in absence of oxygen. I have tested this point in the case of *Ophiopsila aranea* and *Acholoë astericola*, both forms in which oxygen is necessary for luminescence.

I do not believe that the significance of the relation between fluorescence and chemiluminescence is clear at the present time. Zocher and Kautsky (1923) believe that molecules ordinarily excited to luminesce by the energy of a chemical reaction (oxidation) are excited to fluoresce

by the energy of the absorbed ultra-violet radiation. Similarity in spectra would support this view but that does not seem to be always the case (Dufford, Nightingale and Calvert, 1924), and it is more often the oxidation product of the chemiluminescent substance which is fluorescent, than the chemiluminescent body itself. We need further data before an explanation is attempted. That luminous organs of all luminous animals do not show fluorescence may be due to the use of near ultra-violet as the exciting light. Perhaps some forms fluoresce only in far ultra-violet. This point is not easily tested as screens absorbing the visible but transparent to far ultra-violet are not known.

SUMMARY

Tissues of animals and plants that show marked fluorescence in near ultra-violet light are described and a special study made of fluorescence of the photogenic material of luminous animals.

Beroë, Amphiura, Ophiopsila, Acholoë and Copepods show marked fluorescence of the luminous tissue, after a strong luminescence, even when the luminescent light has completely subsided, probably due to fluorescence of the oxidation product of luminescent material. Fireflies, *Pyrophorus* and *Myctophum* always show fluorescence of the luminous organ but this may be due to continual presence of the oxidation product of luminescent material.

Luminous bacteria, fungi (*Panus*), radiolarians, *Pennatula*, *Funiculina*, *Thelepus*, *Tomopteris*, *Pholas*, *Cypridina*, *Heteroteuthis* and *Balanoglossus* show no special fluorescence. *Chaetopterus* and *Microscolex* are doubtful, since their luminous slime is quite fluorescent but non-luminous slime is often fluorescent also.

Many luminous fish preserved in formalin show markedly fluorescent luminous organs. The luminous organ of the fire-fly is fluorescent after boiling and when dried (even after 10 years). *Ophiopsila* and *Acholoë* show fluorescence but not bioluminescence in absence of oxygen.

The color of the fluorescence is about the same as that of the luminescence. This statement is not always true, for the fluorescence of the living fire-fly, *Photinus pyralis*, is very decidedly more yellow than the orange luminescence of this form.

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SPECIFIC INFLUENCE OF THE THYROID GLAND ON HAIR GROWTH[1]

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In connection with the study of hair-falling mechanism occurring in hypothyroidism (cretinism and myxedema) and hyperthyroidism (Simpson, 1922, 1924; Levy-Franckel, Juster and Van Bogaert, 1923), the following experiments on the influence of the thyroid gland upon hair growth have been undertaken.

EXPERIMENTAL. Healthy albino rats which had been raised in the laboratory were used. They were kept hygienically and fed regularly every day.

In all the experiments, age was controlled: in some, sex also. The general scheme was to have in each experiment one animal without thyroids; another was fed with Armour's desiccated thyroid in the dosage of 0.03 gram per capita per day; a third was retained as a weight control i.e., chronic starvation (Chang, 1925); and a fourth, as a normal control. Variation of the above arrangement was made according to the size of the litter. Thyroid was fed to the thyroidectomized and chronically starved animals therapeutically at the end of each experiment.

Milk and bread constituted the control diet. Where chronic starvation was required, this diet was given in insufficient amount so that the body weights of the undernourished animals were kept on the same level as those of the thyroidectomized animals.

Attempt at saving the parathyroids was made in all the thyroidectomy operations. Where such precaution failed and tetany occurred, lactose was resorted to (Dragstedt, 1922).

Reading of hair growth was made from a carefully shaved rectangular area, about 2 by 3 cm., with its left lower corner one phalanx above the left hip. Several new hairs were pulled out by a fine forceps, and measured on a ruler every other day until full growth. The criterion of full growth was based on the perfect covering of the shaved area. In this connection, it should be mentioned that the hairs surrounding the rectangular area had been parted away before shaving; for if they were cut short, they would grow slower than the new hairs and mislead the reading. Area of approxi-

[1] Read before the Institute of Medicine of Chicago, April 23, 1926.

mately the same size and location was shaved in all the animals, shaving being done under light ether anesthesia. As a further precaution, a control reading of hair growth under normal condition was made on each animal before the experiment, so that in each experiment we had both the individual and group comparisons side by side. Moreover, the study of hair growth in each experiment was repeated at least three times on each rat in order to check the result.

RESULTS. Seven experiments have been completed. Table 1 and

TABLE 1

Data of experiment 6

All animals were on liberal control diet except the one on chronic starvation

ANIMAL NUMBER AND SEX	EXPERIMENT CONDITION	AGE AT START OF EXPERIMENT	BODY WEIGHT		DAYS FOR INITIAL HAIR GROWTH AFTER SHAVING	DAYS OF COMPLETE HAIR GROWTH AFTER SHAVING
			Initial	Final		
		days	*grams*	*grams*		
1♂	Control	30	72	155	12	28
	I. 2.25 grams thyroid	101	169	198	3	47
	II. 1.5 grams thyroid	168	211	245	2	40
2♂	Control	30	60	134	6–16	28
	I. Control	101	180	215	9	23
	II. 1.5 grams lactose	168	251	269	14	32
3♀	Control	30	62	127	12	28
	I. 1.03 grams thyroid	101	152	158	3	15
	II. 2.0 grams thyroid	168	168	184	12	24
4♂	Control	30	76	179	12	26
	I. Chronic starvation	101	151	157	25	58
	II. Chronic starvation + 0.75 gram thyroid	168	187	130	10	24
5♂	Control	30	70	165	12	26
	I. Thyroidectomy	101	151	147	33+	?
	+ 0.66 gram thyroid and 7.5 grams lactose		147	156	2	24

figures 1, 2, 3 give data and illustrations for three typical experiments. The results, on the whole, can be given as follows:

1. Effect of thyroidectomy. Thyroidectomy caused a retardation of hair growth. The results obtained from five animals were uniform (animal 5 in table and fig. 1; animal 1 in fig. 3). Where no such result was obtained, incomplete thyroidectomy or pieces of regenerated thyroids were found at autopsy and confirmed by histological study (animal 3 in fig. 3; animal 2 in fig. 2). Typical dry skin was present. There was also a considerable

amount of brownish scales (cholesterol?). Animals did not gain in weight in spite of normal food intake.

2. Effect of chronic starvation. Four undernourished animals with their body weights kept on the same level as those of the thyroidectomized animals in four experiments, showed a similar retardation of hair growth as the thyroidectomized animals (animal 4 in table and fig. 1; animal 4 in fig. 2; animal 5 in fig. 3).

3. Effect of thyroid feeding. Feeding thyroid in proper amount (0.5 to 0.7 gram) to the thyroidectomized animals improved the hair growth (animal 5 in table and fig. 1; animal 1 in fig. 3). Curiously enough, it produced the same effect on the chronically starved animals even though their body weights were decreased further by the increased catabolism in addition to the originally insufficient anabolism (animal 4 in table and fig. 1; animal 4 in fig. 2; animal 5 in fig. 3).

To some of the normal animals, feeding of small amount of thyroid accelerated the hair growth above the normal reading (animal 3 in table and fig. 1); while in others, no such result was obtained (animal 1 in table and fig. 1). Feeding of large amount of thyroid brought about a definite retardation of hair growth in three animals. This was found to be due to the secondary nutritional disturbance (animal 3 in fig. 2).

The coating produced by thyroid feeding in all the animals under all conditions was very characteristic. It was soft, smooth and glossy, consisting of short hairs. Even in case where no acceleration or improvement of hair growth was obtained, such a coating was present.

The posture of the thyroid-fed animals was also significant. They preferred to lie down most of the time with their limbs completely stretched out, and oftentime on their back to expose the ventral surface. This was due presumably to the increased body temperature produced by thyroid feeding, for normal animals put in the incubator or under the sunshine would show similar postures.

4. Effect of lactose. Lactose in the amount for the aciduric diet to control the tetany, i.e., from 0.5 to 1 gram per capita per day did not show any effect upon the hair growth (animal 2 in table and fig. 1).

Discussion. It has been suggested by several investigators that the thyroid may have some influence on the hair growth. Furuya (1924), working in Asher's Laboratory, found a delayed hair growth in the thyroidectomized rabbit. Clinical cases of dystrophies of hair and nails with hereditary occurrence of hypothyroidism have been reported by Nicollé and Hallipré (1895), White (1896), Hoffman (1908), Eisenstaedt (1913) and Barrett (1919).

As far as we are aware, however, none has given a set of well-controlled experimental evidences showing the specific action of thyroid upon hair growth; for it is rather difficult to differentiate most of the symptoms

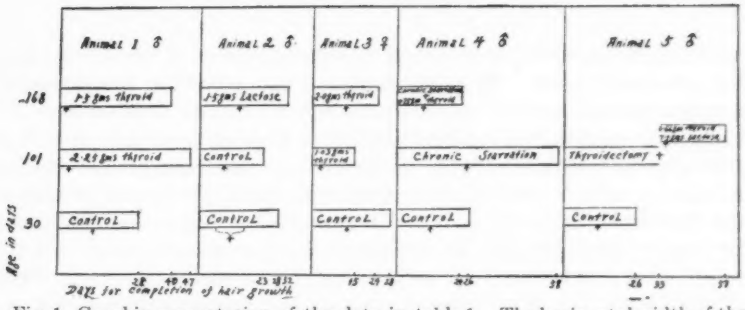

Fig. 1. Graphic presentation of the data in table 1. The horizontal width of the rectangular block under the various animal columns represents accurately in days the length of time necessary for the individual animals to attain the full growth of hair in the shaved area. Examination of the width of the blocks allows a direct comparison of the difference in the rate of hair growth in the various animals under different experimental conditions. Group comparison horizontally; individual comparison longitudinally. ↑ indicates the date of initial hair growth; ⌒ denotes the initial hair growth followed by a stationary period.

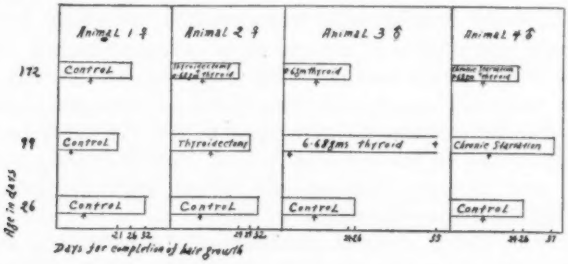

Fig. 2. Graphic presentation of the data of experiment 3. All animals were on liberal control diet except the one on chronic starvation. Legend of figure 1 applies.

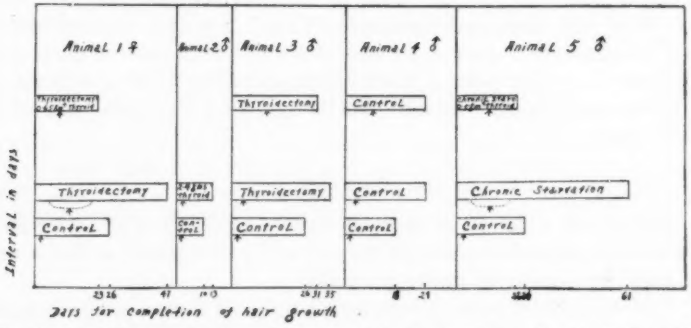

Fig. 3. Graphic presentation of the data of experiment 2. The birth date of this litter was missing, but the intervals between the readings are accurately represented. All animals were on liberal control diet except the one on chronic starvation. Legend of figure 1 applies.

occurring in thyroid disturbances from those produced by the accompanying nutritional upset. That retardation of hair growth in the thyroidectomized animals, and its improvement by the thyroid feeding does not necessarily suggest the specific function of thyroid upon hair growth is evident; for, in the first place, undernutrition itself can produce similar retardation of hair growth, and undernutrition, to be sure, is present in the thyroid disturbances; and, in the second place, improved hair growth by thyroid feeding might be the result of improved nutrition. On the other hand, undernutrition induces thyroid atrophy (Jackson, 1916; Chang, 1925). So whether the thyroid or the undernutrition is the primary cause for the hair reaction seems to be difficult to determine. However, our experimental finding that thyroid feeding to the animals on chronic starvation diet does improve the hair growth in spite of the further decreased body weight is, we believe, a significant proof for the specific influence of the thyroid gland on hair growth.

Two possible explanations might be given for the lack of response of some of the normal animals to thyroid feeding. First, the individual variation of the thyroid might be responsible. Secondly, in this qualitative study, the amount of thyroid given is not controlled. Where large amount of thyroid has been fed, there is definite retardation of hair growth due to secondary nutritional disturbance of excessive catabolism.

The smooth, soft and glossy coating of the thyroid-fed animals is interesting in view of the fact that in birds thyroid feeding induces typical feather and plumage (Horning and Torrey, 1923; Torrey and Horning, 1922, 1925; Zavadovsky, 1925).

With such results at hand, one seems to be justified to conclude that thyroid does have a specific influence on hair growth; but the questions arise: What constituent of the thyroid is responsible for the hair reaction? Could it be iodine as perhaps might be inferred from the works of Smith (1917, 1919) and Hart and Steenbock (1918)? Could it be tryptophan? Is it a hormone or a general protein reaction? Experiments have been in progress to answer some of these questions in the hope of determining whether or not this hair reaction can be used as a functional test of the thyroid gland.

SUMMARY

1. Albino rats deprived of thyroids show a retardation of hair growth. Upon feeding suitable amount of thyroid (0.5 to 0.7 gram) in the dose of 0.03 gram per day, hair grows normally.

2. Undernourished animals with intact thyroids show a similar retardation of hair growth. Thyroid feeding to such animals kept on the chronic starvation diet improves the hair growth in spite of the further decreased body weights caused by excessive catabolism in addition to the originally

deficient anabolism. This proves the specific influence of the thyroid gland on hair growth.

3. Feeding of thyroid accelerates the hair growth in some normal animals, while in others, no such result was obtained. Further experiments are required to determine whether the refractory animals have thyroids functioning at a rate optimum for hair growth.

4. Excessive amount of thyroid given by repeated administrations over a long period of time retards the hair growth of the normal animal due to the secondary nutritional upset of increased catabolism.

5. Further work on the study of the constituents of the thyroid gland responsible for this hair reaction has been in progress in order to determine whether or not this hair reaction can be used as a functional test of the thyroid gland.

The writer thanks Dr. A. J. Carlson for his guidance and aids during the investigation and in the preparation of the manuscript; and Dr. A. B. Luckhardt for his encouragement and suggestions. He is also indebted to Mr. Y. H. Ho for his suggestions in the arrangement of the table.

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HEPARIN

III. Effect on Coagulation Time when Added to Blood after Clotting has begun[1]

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The action of heparin in delaying coagulation of shed blood was first described by Howell and Holt (1918) and the possible mechanism of its action was discussed by Howell in a later paper (1925). Its intravascular action was demonstrated in experiments described in a previous paper (Reed, 1925). It is the purpose of this paper to report results of experiments in which heparin was added to shed blood at various intervals after drawing and at various stages after coagulation had begun.

Both rabbits and dogs were used. A quantity of blood was drawn from a normal unanesthetized animal and 1 cc. was added to each one of a series of small test tubes. Number 1 of each series was always used to determine normal coagulation time. In number 2 was placed 0.1 cc. of a solution of heparin dissolved in Ringer solution of such concentration that this volume contained 1 mgm. of heparin, except in certain experiments when another concentration was used as stated in the following protocols. To each of the other tubes was added at varying intervals a like quantity of heparin.

The following brief reports of some of the experments will show the effect of heparin on partially coagulated blood.

Experiment 1. Rabbit, normal coagulation time 4 minutes, 15 seconds. Additions of heparin at 1 minute intervals. Tubes 2 and 3 did not coagulate in 5 hours. No. 4 coagulated in 110 minutes while no. 5, to which heparin was added 4 minutes after drawing, coagulated in 4 minutes and 30 seconds, a delay of 15 seconds.

Experiment 2. Rabbit, normal time 2 minutes, 30 seconds. No. 2 did not clot in 2 hours' observation. No. 3, to which heparin was added 2 minutes after drawing, coagulated in 12 minutes.

Experiment 7. Dog, normal time 8 minutes. No. 2 began to sediment in 6 minutes. Heparin added to 6 additional tubes at 1 minute intervals beginning 2 minutes after drawing. In none of these was there any sign of coagulation in 24 hours but all were completely hemolyzed.

Experiment 8. Dog, normal time 5 minutes, 30 seconds; 0.4 mgm. of heparin added to each tube. Nos. 2 and 3 did not clot at all. No. 4, to which heparin was

[1] The expenses of this investigation were borne in part by a grant from the Research Committee of Phi Rho Sigma Medical Fraternity.

added 4 minutes after drawing, coagulated in 12 hours. No. 5 received heparin after 5 minutes, clotted in 9 minutes.

Experiment 10. Dog, normal time 5 minutes; 0.2 mgm. heparin added to each tube. Nos. 2 and 3 unclotted and not sedimented in 4 hours but found firmly coagulated when next observed 15 hours later. No. 4 received heparin 3 minutes after drawing, coagulated in 45 minutes. No. 5 received heparin 4 minutes after drawing, coagulated in 27 minutes.

Experiment 13. Dog, normal time 4 minutes, 45 seconds; 0.1 mgm. heparin added to remaining tubes at 1 minute intervals beginning 1 minute after drawing. No. 2 coagulated 24 minutes; no. 3, 22 minutes; no. 4, 10 minutes; no. 5, 5 minutes.

Experiment 14. Dog, normal time 4 minutes, 30 seconds; 0.1 mgm. heparin added at 1 minute intervals to remaining tubes except no. 4 which received 0.2 mgm. No. 2 showed thin film of coagulum on surface of tube in 1 hour; found firmly clotted 16 hours later. No. 3 had already shown thin film on surface of tube when heparin was added; no further change in 2 hours, 30 minutes; firmly clotted 15 hours later. No. 4 (cf. supra) received heparin 2 minutes after drawing; had already shown film on surface of tube but no further change in 15 hours. No. 5 same as no. 3.

Experiment 15. Dog, normal time 5 minutes, 30 seconds; 0.05 mgm. heparin added to remaining tubes at 1 minute intervals. No. 2 showed marked sedimentation in 10 minutes, coagulated 75 minutes. No. 3, 33 minutes; no. 4,15 minutes. No. 5 received heparin 4 minutes, 30 seconds after drawing coagulated 11 minutes.

Experiment 19. Dog, normal time 9 minutes; 1 mgm. heparin added to no. 3 in 2 minutes, no. 4 in 4 minutes and no. 5 in 7 minutes after drawing. Nos. 2, 3 and 4 never clotted; no. 5, 12 minutes.

Experiment 22. Dog, normal time 5 minutes; 1 mgm. heparin added to no. 3 in 2 minutes, to no. 4 in 3 minutes and to no. 5 in 3.5 minutes after drawing. No. 2 showed very little sedimentation in 30 minutes and did not coagulate at all. Nos. 3 and 4 showed fine fibrin shreds attached to the surface of the tubes in 1 hour but very little sedimentation in 2 hours and no further change in 24 hours. No. 5 coagulated in 33 minutes.

It is apparent from these experiments and many others showing similar results that, whatever the mechanism of action of heparin, even after the process of coagulation has begun, and certainly after the changes preliminary to demonstrable coagulation have begun, the addition of relatively small amounts of heparin may prolong coagulation time to a marked degree or even arrest that process entirely.

As a rule, heparinized blood will undergo rapid sedimentation but this does not always occur, even in blood that remains fluid until hemolysis occurs. In some instances heparinized blood will sediment so rapidly and completely that subsequent centrifugalization will not separate corpuscles and plasma any more completely. The reason for the variations in this respect of samples of blood from different animals is not now apparent.

Thanks are due Hynson, Westcott and Dunning who generously supplied a part of the heparin used in these experiments.

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ELECTRICAL CONDUCTIVITY, ELECTRICAL POTENTIAL AND HYDROGEN ION CONCENTRATION MEASUREMENTS ON THE SUBMAXILLARY GLAND OF THE DOG RECORDED WITH CONTINUOUS PHOTOGRAPHIC METHODS

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From the structure and chemical composition of living matter it seems inevitable that any change in function or condition of a tissue must be accompanied by electrical phenomena. It is only necessary to observe stringent precautions against the introduction of extraneous variables to show the effects of numerous variables on the characteristics of the action current of the submaxillary gland of the dog. It has been shown (1) that stimulation of the chorda tympani of equal strength, of equal duration, repeated at equal intervals provoking equal secretion, produced superimposable electrical deflections approaching in similarity a series of action currents of the regularly beating heart of the normal individual. Introducing any single variable, such as strength of stimulation, duration of stimulation, duration of rest, altered blood supply, resistance to the flow of saliva produced characteristic changes in the electrical behavior of the gland.

Though the results on the whole were uniform they offered very little clew to their interpretation. Hoping to advance this subject further we have used accessory methods of approach. Since the solution of the problem appears to hinge primarily on an understanding of the processes occurring at the cell membrane it seemed that a study of changes in electrical resistance[1] might prove of value. The effects of the highly mobile hydrogen ion on the development of electrical potentials suggested the study of changes in the hydrogen ion concentration of the venous blood flowing from the gland as well.

METHOD. All experiments were performed on dogs, anesthetized with hypodermic injection of 10 mgm. morphine sulphate per kilogram body weight followed in 20 minutes by a rectal injection of 0.8 gram urethane per kilogram body weight. The gland was dissected free of outlying fascia to

[1] Confusion frequently arises as a result of employing the term "conductivity" for both electrical and physiological conductivity. It therefore seems desirable to use "electrical resistance" when referring to the former quantity and to reserve "conductivity" for use when referring to the transmission of a physiological disturbance.

permit direct application of electrodes—thus minimizing electrical resistance in circuit. The chorda lingual and vago-sympathetic nerves were exposed and precautions for uniform stimulation observed (1). A glass cannula was inserted high in Wharton's duct. All branches of the external jugular vein with the exception of those draining the gland were ligated to permit measurements of the volume-flow of blood from the external jugular vein.

The gland was usually excited by tetanic stimulation of its nerves. In the early part of the work the stimulating current was furnished by an induction coil. Due to the difficulty of correctly ascertaining the strength of the secondary current, and the annoying induced disturbances inherent in this form of stimulus, it was replaced by 60 cycle alternating current. The reduced stimulating voltage was read on the alternating current voltmeter.

Salivary secretion was recorded photographically by the opaque drop method. The secretion was led into a system of two displacement bottles—the first containing mineral oil, the second opaque ink. The saliva displaced the mineral oil which in turn displaced the ink, this falling from a tube immersed in a vessel of oil immediately in front of the slit of the camera. This method slows the falling shadow of the drop sufficiently for photographic registration and by greatly increasing the size of the drop it produces a legible record of a rapid secretion. The volume-flow of blood was recorded by the same method, the blood dropping directly in a separate vessel of mineral oil.

The hydrogen ion concentration of the venous blood was recorded with the use of the manganese dioxide electrode (2). The changes in E.M.F. resulting from changes in acidity were photographed with the use of the string galvanometer. The advantage of employing a vacuum tube in circuit with the electrode occurred to us in that it draws no current and therefore avoids polarization of the electrode. This method proved satisfactory but in the animal experiments there was some difficulty encountered in avoiding induced effects of the stimulating current. This led us to use the simpler method of connecting the string directly in series with the electrode. Due to the high resistance of the string, the fact that the string was fully compensated before every observation, and that we were interested primarily in qualitative results, the method served our purpose.

Zinc-zinc sulphate non-polarizable electrodes used early in the research for recording electrical potential and electrical resistance were soon replaced by La Picque electrodes of disc design, 5 mm. $\times$ 5 mm. square. The discs were wrapped in a thin layer of absorbent cotton and sewed in place on the gland. This procedure insures a constant position of the electrode with respect to the gland and permits closure of the wound, thus preventing disturbances through changes in temperature and drying.

The electrical deflections were recorded by means of a d'Arsonval galvanometer[2] operated potentiometrically by the gland through a vacuum tube type UV 201. When that surface of the gland to which the grid was connected became positive with respect to the other surface which was connected to the filament, the plate current increased and vice versa.

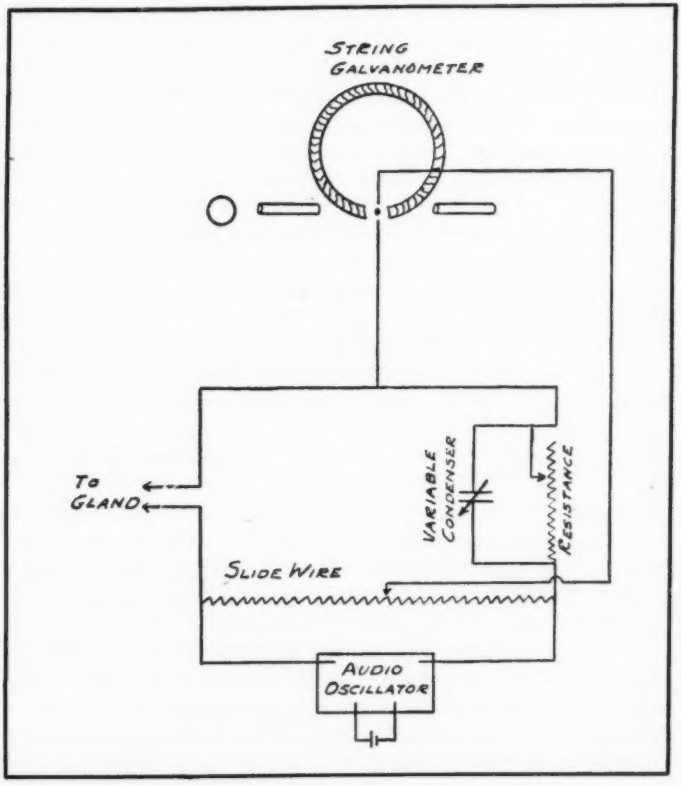

Fig. 1. String galvanometer as resistance recorder

There was, accordingly, a fluctuation of current through, and a variation of potential across, a 150,000 ohm resistance in the plate circuit. Inasmuch as the galvanometer was connected across the ends of the resistance it was this magnified potential variation that caused the deflection. A potenti-

[2] This galvanometer had a sensitivity of 13,000 megohms (7.7×10^{-11} amperes), a period of 14 seconds, resistance of 570 ohms, and a critical damping resistance of 22.000 ohms. It was at a distance of 105 cm. from the camera.

ometer in series with the latter made it possible to balance the constant plate current and thus keep the galvanometer compensated. In addition to amplification of the electrical deflections the vacuum tube offered the distinct advantage of the use of the same electrodes for recording both changes in electrical potential and electrical resistance.

So far as we are aware, no suitable method has been employed for recording changes in electrical resistance. The simplest and most direct method of substituting a string galvanometer for the telephone in the classical bridge method was tried, figure 1. A change in resistance registers as a change in width of the shadow of the vibrating string. This method—particularly if the unbalance were amplified—should prove valuable but the advantages of the second method will be apparent.

This consists of an alternating current bridge, a three-stage thermionic amplifier, and a direct current Wheatstone bridge. The three are so combined that a change in conductivity of the tissue under study alters the balance of the alternating current bridge and consequently the input voltage to the amplifier. This variation of input voltage changes the conductance of the last tube, the plate to filament circuit of which is one arm of the direct current bridge. The result is a deflection of the direct current recording galvanometer placed in this second bridge circuit.

Figure 2 gives the details of the circuit. The gland, or other tissue, is placed in one arm of the alternating current bridge; a variable resistance, *A*, and complementary portions of a slide wire resistance, *C*, comprise the remaining three arms. The source of alternating current is a 1000 cycle oscillator, *B*, of the commercial type. The telephone usually employed for detecting the bridge balance is replaced by the primary of an audio frequency transformer, *D*, the secondary being across the grid and filament of a type UV199, or other suitable thermionic tube. The grid of this tube is maintained at the proper potential by means of the battery, *F*, and the potentiometer, *G*. The plate circuit of this first tube consists of a 150,000 ohm resistance, *H*, and a 90 volt storage battery which also furnishes the plate voltage for the other two tubes. One end of the high resistance, *H*, is connected to the grid of the second tube through a 0.01 microfarad condenser, *I;* the other side to the filament. The 5 megohm resistance, *J*, affords a high resistance escape for the electronic charge which tends to accumulate on the grid. This second tube is coupled to the third by means of an audio frequency transformer, the grid of the third tube being isolated by a 0.01 microfarad condenser shunted by a 5 megohm resistance. The plate to filament circuit of this last tube is, as has been stated, one of the four arms of the direct current bridge; the other three are the 25 ohm fixed resistance, *K*, the 25 ohm variable resistance, *L*, and the 12,000 ohm fixed resistance, *O*. This bridge contains as the recording element a d'Arsonval galvanometer with a sensitivity of 1 megohm and a period of

less than a second. A beam of light is reflected from the galvanometer mirror onto the slit of a moving film camera so that a continuous photographic record is obtained of resistance variations. When records of very rapid changes in resistance were desired a string galvanometer was used in place of the d'Arsonval for recording purposes.

The theory of operation of the system is this: When the alternating current bridge is perfectly balanced none of the 1000 cycle current flows through the primary of the first transformer. The amplifier is consequently in equilibrium and, if the direct current bridge is balanced by the variable resistance, L, the galvanometer will be at its rest position. If, now, the conductivity of the gland changes, the alternating current bridge becomes unbalanced and a certain amount of the 1000 cycle current flows through the primary of the transformer. This causes the potential of the

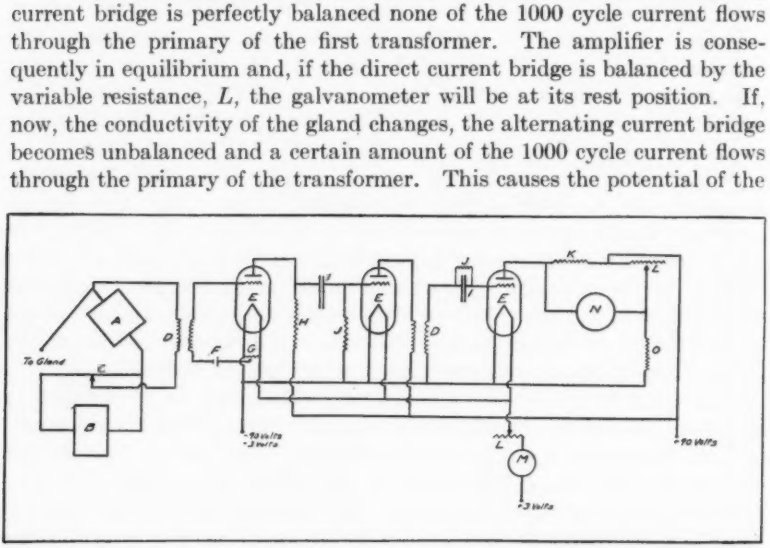

Fig. 2. Vacuum tube resistance recorder. A—variable resistance; B—audio oscillator; C—slide wire resistance; D—audio frequency transformer; E—vacuum tube; F—grid battery; G—potentiometer; H—150,000 ohm resistance; I—0.01 M.F. condenser; J—5 megohm resistance; K—25 ohm resistance; L—25 ohm variable resistance; M—ammeter; N—galvanometer; O—12,000 ohm resistance.

grid of the first tube to oscillate about its normal value, producing consequent variations of the same frequency in the plate current. Due to this alternating current through the 150,000 ohm resistance, there is an alternating voltage applied to the grid with respect to filament of the second tube of a larger value than that applied to the first. This voltage, in turn, causes the current through the primary of the second transformer to oscillate at 1000 cycles and so induces an electromotive force in the secondary very much larger than that originally derived from the alternating current bridge. During the positive half of each cycle of this amplified voltage an increased number of electrons are drawn to the grid of the third tube and, due to the action of the grid condenser, this charge is not entirely lost during the negative half of the cycle. The result is an increased

negativity of the grid relative to the filament and a consequent decrease in the plate to filament conductance. The direct current bridge is therefore unbalanced and the galvanometer deflected.

It is evident that either an increase or decrease in tissue resistance causes an increase in 1000 cycle current through the in-put transformer and a deflection of the galvanometer that is in the same direction in both cases. The following procedure was therefore employed. The reading of the galvanometer was noted for a balanced alternating current bridge; the bridge was then unbalanced by varying the ratio arms a known amount and in such a way that an increase in gland resistance caused the galvanometer to return toward its balance position. In this way the sign of the resistance variation was indicated by the direction of the galvanometer swing and the magnitude of the change was easily calculated. The following example illustrates the method. The bridge was balanced and the resistance of the gland was found to be 180 ohms. The bridge was then unbalanced to such an extent as would correspond to an increase in gland resistance of 25 ohms; the corresponding galvanometer deflection was 9.5 cm. On stimulation of the gland the galvanometer swung back 8.5 cm. toward its balance position, an increase in resistance of $8.5/9.5 \times 25$ ohms $= 22.4$ ohms or $22.4/180 = 12.4$ per cent.

The above computation is made on the assumption that there is a linear relationship between resistance change and galvanometer deflection. Our results show that such is practically the case provided the resistance does not increase sufficiently to bring the bridge almost into balance.

This system has a number of desirable features by virtue of which it should serve as an instrument for opening up numerous fields of physiological investigation. It gives a continuous record of changes in resistance that makes it possible to follow photographically the variations in permeability of a tissue or organ. The duration of resistance change to which it will respond is limited mainly by the rapidity of the recording instrument; in conjunction with a string galvanometer it will therefore follow exceedingly rapid fluctuations. By making the amplification factor large it will have a high sensitivity and will therefore be suitable for precise work. The circuit is such that it avoids the slow drift inherent in many amplifying systems inasmuch as the transformer coupling between the second and third tubes passes only the high frequency variations.

Results. The results reported in this paper have been taken from experiments performed on twenty-eight animals and in many of the experiments a number of tests were made of each point investigated.

Certain general results of activity of the submaxillary gland may be pointed out before going on to the more detailed experiments. We have confirmed many of the previous observations regarding electrical deflections (1), (3), (4), (5). Among these may be mentioned the invariable

presence of a negative deflection[3] when there is secretion produced by other than stimulation of the cervical sympathetic fibres and a positive deflection in the latter case.

Excitation of the gland sufficient to produce secretion, whether by electrical stimulation of either the cranial or cervical sympathetic nerves or by the injection of pilocarpin, caused an increase in the resistance of the gland. This increase followed the beginning of stimulation by about three seconds and frequently preceded visible secretion by several seconds or more. It generally long outlasted the period of stimulation and secretion.

Secretory activity always caused an ultimate increase in the acidity of the blood flowing from the gland. This increase in the acidity of the blood generally reached a maximum after secretion was nearly over, certainly after the height of glandular activity was passed. The exact time at which this effect was produced in the blood cannot be stated, however, due to the difficulty in determining the time that elapses between the instant the blood leaves the gland and its arrival at the electrode vessel. In certain cases

Fig. 3. Occlusion of Wharton's duct. *S*—secretion in drops; *ST.*—stimulation of chorda tympani by 1 volt A.C.; *E.R.*—electrical resistance; *E.D.*—electrical deflection (negative components upwards); *T*—time in 15 seconds; *1*—occlusion of Wharton's duct; *2*—deocclusion of duct; *3*—occlusion of duct.

STIMULUS	RESISTANCE INCREASE IN PER CENT	E.D. IN MILLIVOLTS	pH (ARBITRARY UNITS)	SECRETION
1—Deoccluded	12.4	−0.192	−10	21
2—Occluded	16.0	−0.218	11;−18	
3—Deoccluded	12.8	−0.312	3;−9	28
4—Deoccluded	12.8	−0.182	2;−9	21
5—Occluded	14.6	−0.234	10;−18	

Fig. 4. Occlusion of carotid artery. *S*—secretion in drops; *ST.*—stimulation of chorda tympani by 1 volt A.C.; *E.R.*—electrical resistance; *T*—time in 15 seconds; *1*—occlusion of carotid artery; *2*—deocclusion of artery; *3*—occlusion of artery.

STIMULUS	RESISTANCE INCREASE IN PER CENT	pH (ARBITRARY UNITS)	SECRETION
1—Deoccluded	11.2	−12	10
2—Occluded	14.7	−49	16
3—Deoccluded	12.0	−19	15
4—Occluded	15.2	−41	15

[3] A negative deflection indicates the outer surface of the gland to be electrically negative to the hilus surface and is represented as an upward movement of the curves, Gesell (1). This was the lead employed for resistance measurements as well as electrical deflection.

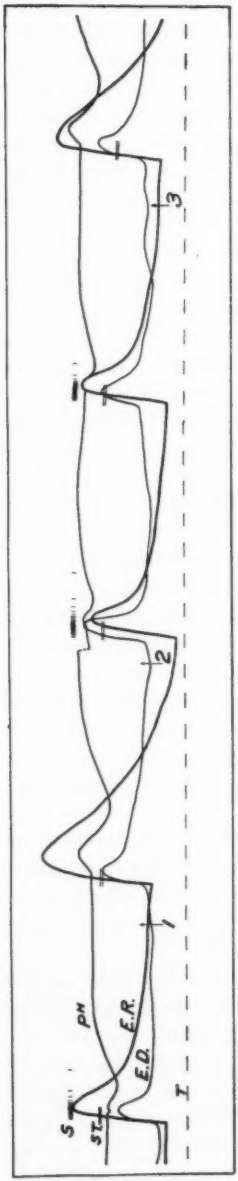

Fig. 3

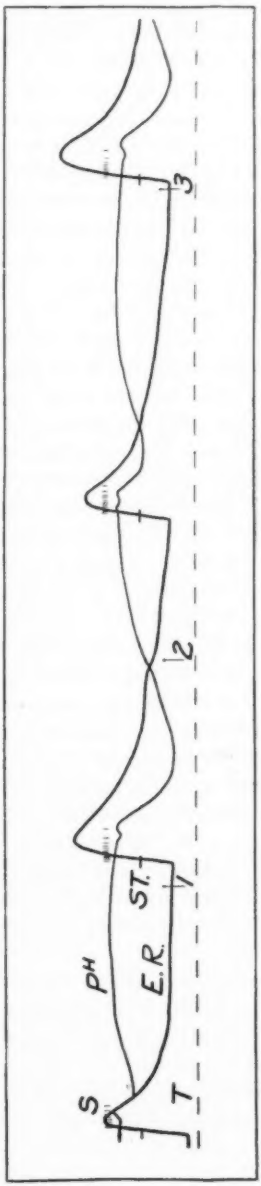

Fig. 4

there were components present in this variation other than the simple acid movement. The most prominent of these was a rather large decrease in acidity that came during, or shortly after, stimulation.

Occlusion of Wharton's duct. In studying the mechanism of secretion it seemed desirable to investigate the effect of secretion against pressure. This was done by occluding Wharton's duct. Stimulation of the chorda tympani with the duct closed is referred to as an occluded stimulus and with the duct open as a deoccluded stimulus. Characteristic results are given in figure 3 and the accompanying table.

The occluded stimuli invariably caused a greater resistance increase than the deoccluded stimuli. If the occluded stimulus was strong enough and of sufficient duration, the resistance subsequently dropped to below its pre-stimulus value. Such an after effect never resulted from stimulation with the duct open. The drop in resistance as a result of stimulation with the duct occluded was often associated with a decreased response of the gland to the following deoccluded stimulus. This was especially evident toward the end of a series and was frequently apparent in the decreased magnitude of secretion as well as resistance variation.

In eight out of ten experiments the principal component of the electrical deflection (-2) was larger as a result of a stimulus with the duct occluded than with it open. Figure 3 shows one of the two exceptions.

In every experiment the occluded stimulus produced the greater increase in acidity of the blood. Usually the alkaline component was also larger as is evident in the figure. The blood reached its maximum acidity later with occlusion and the return to the pre-stimulus value was less prompt. In a series of stimuli the change in acidity frequently became progressively greater; the electrical deflection smaller.

If there was an escape of dammed-up saliva following deocclusion there was a rapid drop in resistance of a few per cent and sometimes a small negative electrical deflection.

Occlusion of the carotid artery. In these experiments we followed the results of changes in blood supply to the gland resulting from clamping the common carotid artery during stimulation. The stimuli are also referred to as occluded and deoccluded stimuli. A record of such a series is shown in figure 4.

The resistance increased more as a result of an occluded stimulus and the rate of recovery was much slower. These results were obtained invariably although it was necessary that the artery remain occluded in order that the recovery be retarded. Similarly, the increase in acidity of the venous blood was always much larger as the result of an occluded stimulus; the maximum acidity was reached later and the return to pre-stimulus level was less rapid.

A statement as to the effect of reduced blood supply on the magnitude and form of the electrical deflections is rather precarious. In five out of eight series the magnitude of the principal component was larger as the result of an occluded stimulus but in the remaining three the results were inconclusive.

Injection of pilocarpin. This method of stimulation was particularly useful when studying the effects of prolonged secretion, as illustrated in figure 5, A and B. The injection of pilocarpin caused a sustained secretion of saliva sometimes lasting as long as half an hour. This was accompanied by a prolonged increase in resistance. The resistance increase began from five to eighty seconds before there was visible secretion but so far we have not noted an increase in resistance without an ultimate secretion. Generally, if the resistance increased very rapidly it was followed within five to ten seconds by a rapid secretion; if, on the other hand, the resistance increased but slowly, the secretion did not commence until after a comparatively long interval, and was then rather scanty. These points are admirably shown by a comparison of figure 5, A and B. In A the resistance increase was rapid and the secretion which followed within a few seconds was copious. The opposite was true as a result of the first injection in B. In this case the rather slow secretion did not begin until after the resistance had already come to constancy at the increased level.

Pilocarpin secretion was associated with a small negative electrical deflection and an increase in the acidity of the venous blood.

If the duct was occluded during pilocarpin secretion there was a marked fall in resistance and the blood became more alkaline. On deocclusion these effects were reversed, as is shown in the figure. Occlusion of the artery caused a slowing of secretion and tended to increase the acidity of the blood. Stimulation of the vago-sympathetic fibres produced an initial, very slight increase in the rate of secretion that was followed by a prolonged and considerable decrease. The accompanying resistance increase long outlasted the temporarily accelerated secretion.

A paralyzing injection of atropin stopped secretion and was accompanied by a gradual decrease in resistance and subsequent stimulation of the chorda tympani produced no variation in resistance, blood acidity, or electrical potential regardless of the strength of stimulation. Such a stimulus without response is shown in figure 5 B.

Effects of asphyxia. Seven experiments were done on the effects of asphyxia in the hope that they would throw some light on the relationship between the acidity of the blood and glandular activity. Under the conditions of these experiments increased acidity of the blood had no effect upon either secretion or upon the electrical deflections. The resistance of the gland, however, did undergo a small but definite decrease during asphyxia.

This effect is shown in figure 6. The record was taken with the gland in the resting condition and shows the characteristically slow fall and rise of resistance accompanying asphyxia and the recovery. Because of the great importance of any general relationship between blood acidity and cell permeability this phase of the investigation is being extended to other tissues.

Stimulation of the vago-sympathetic fibres. Electrical stimulation of the cervical sympathetic fibres in the vagus produced a small increase in the resistance of the gland. The increase and recovery were, however, very much slower than those resulting from chorda tympani stimulation. Even very strong stimulation failed to produce the larger changes in resistance that accompanied stimulation of the chorda tympani. The scanty secretion and positive electrical deflection reported by other investigators have been observed.

Figure 7 gives a comparison of the change in resistance resulting from stimulation of the vago-sympathetics and the chorda tympani. Although the former stimulus was longer and twice as strong the resistance increase was very much less. Figure 8 shows the effects of vagal stimulation on acidity as well. Note the greatly delayed and slow increase in resistance and acidity as compared with the prompt and rapid effects that were always produced by similar stimuli applied to the chorda tympani.

DISCUSSION. *Relation of resistance changes to electrical deflections.* In planning this work it had seemed reasonable to assume that the resistance changes of the gland would help to explain the electrical deflections because of the obvious dependence of bioelectric potentials on cell per-

Fig. 5. Injection of pilocarpin. *S*—secretion in drops; *E.R.*—electrical resistance; *E.D.*—electrical deflection (negative components upwards); *T*—time in 15 seconds.

	RESISTANCE INCREASE IN PER CENT	E.D. IN MILLIVOLTS	pH (ARBITRARY UNITS)
Fig. 5 A			
1—$\frac{1}{8}$ grain pilocarpin	8.4	−0.037; 0.032	−40
2—Occlusion of duct	−1.5	−0.035; 0.035	12
3—Deocclusion of duct	2.8	−0.030	−13
4—Occlusion of artery	−2.1	0.032	−17
Fig. 5 B			
1 and 2—$\frac{1}{8}$ grain pilocarpin	9.5	−0.010	−11
3—Occlusion of duct	−6.0	−0.030	11
4—Deocclusion of duct	6.0	0.030	−11
5—Atropin	Decrease		Decrease

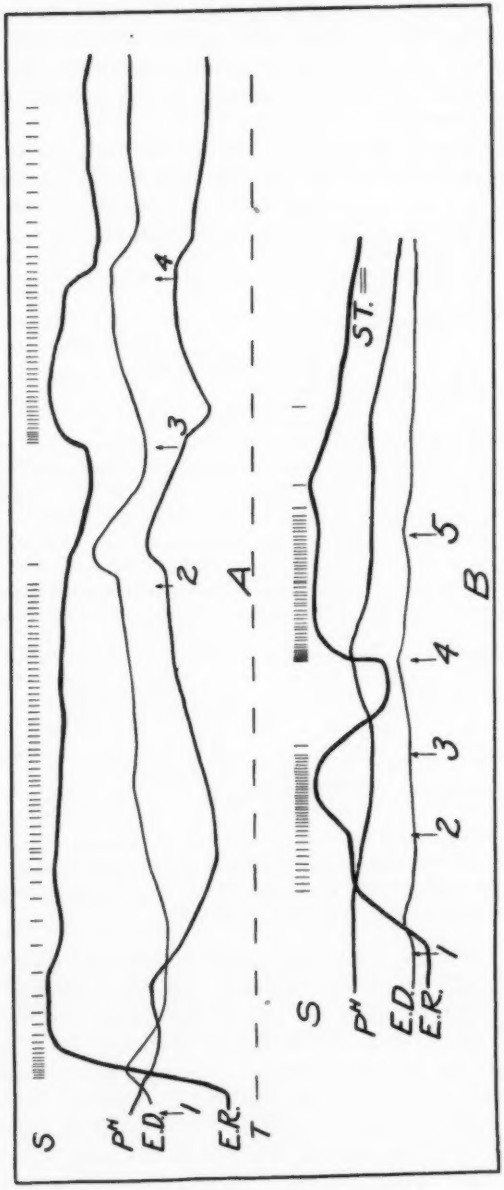

Fig. 5

meability. One of the most striking points of our work was the constancy of behavior of the gland with respect to resistance changes as compared with the variability of the electrical deflections under like conditions. A constancy of electrical deflections was obtained only by observing the most stringent precautions.

Undoubtedly one of the reasons for the greater difficulty in controlling the form of the electrical deflections is that they are the algebraic sum, the resistance changes the arithmetic sum, of the contributions from the several cells. The consequences of this difference may be brought out by

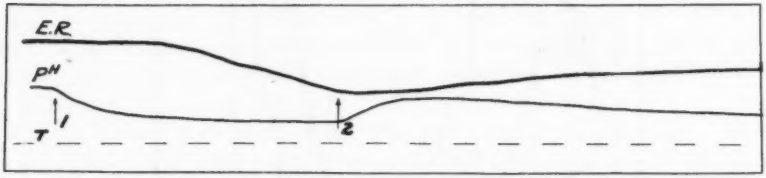

Fig. 6. Asphyxia. *E.R.*—electrical resistance; *T*—time in 15 seconds

	RESISTANCE CHANGE	pH (ARBITRARY UNITS)
1—10 per cent CO_2	3.2 per cent decrease	−20
2—Room air	3.0 per cent increase	+15

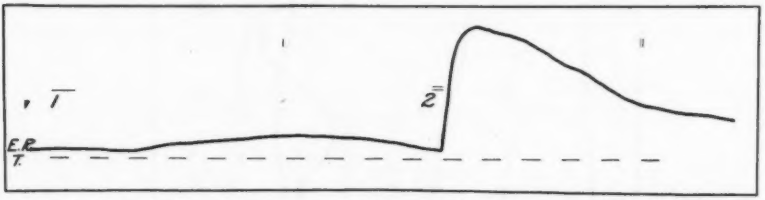

Fig. 7. Stimulation of vago-sympathetics and chorda tympani. *E.R.*—electrical resistance; *T*—time in 15 seconds; *1*—stimulation of vago-sympathetics; *2*—stimulation of chorda tympani with weaker stimulus than in 1.

a consideration of figure 9 which is a simple diagrammatic section through two different acini. The arrows represent the magnitude and sign of the contributions made by two of the cells to the change in potential difference between the leading-off electrodes resulting from stimulation. It is evident that the electrical deflection given by acinus A will be different from that given by acinus B, the sign of the deflection being reversed. It therefore follows that the electrical deflection given by a gland must be a complex function of the position of the leads, the magnitude of the part played by the various cells and their orientation, and the like. But the

resistance across these two acini involves only the magnitude, not the sign, of the various cellular contributions. If then the lengths of the lines represent the effective changes in resistance of the cells, the net resistance change of the two acini between the electrodes will be the same. In general, the resistance of the gland will be a very much simpler function of the position of the leads and the arrangement and condition of the individual cells. Consider, for instance, the effects of gland cells behaving as we know the component fibres of muscles do, certain cells resting while others carry on their active function. For the purpose of illustration let us make the simple assumption that all of the cells are arranged as in acinus A or B. If now eighty per cent of the secreting cells are arranged

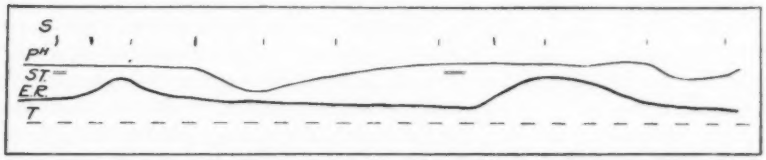

Fig. 8. Stimulation of vago-sympathetics. *S*—secretion in drops; *ST.*—stimulation of vago-sympathetics with 10 volts A.C.; *E.R.*—electrical resistance; *T*—time in 15 seconds.

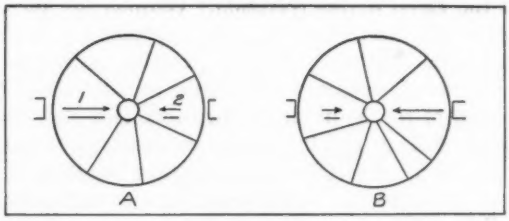

Fig. 9

as in acinus B, the potential difference will be directed from right to left; if at another instant sixty per cent are as in B and the other forty as in A, the electrical deflection will still be in the same direction but much smaller due to the balancing of oppositely directed potential gradients; while if at another time twenty per cent are as in B and eighty as in A, the size of the deflection will again be increased but will now be directed from left to right. The resistance change on the other hand will be the same in each case.

If the all-or-none-law holds for gland cells it undoubtedly contributes to the difficulty of controlling the electrical deflections. If a stimulus is delivered which is below the threshold for cell 1 but above that for cell 2, the potential difference variation will be directed from right to left,

whereas with a stronger stimulus the potential difference will act from left to right. An increasing stimulus would thus alter the sign and form of the electrical deflection but would have no effect on the resistance changes other than a greater increase in net resistance. Due to the very large number of cells which enter into the response of the gland there is no doubt but that extreme irregularities in the electrical deflections would tend to be ironed out by the averaging effect. Nevertheless there is still a possibility that such causes may account for the differences in form of response given under slightly different conditions.

Significance of the resistance changes. Before attempting any interpretation of the observed increase in resistance is it to be emphasized that vasomotor effects are not the determining factors. That the resistance changes are due to the activity of the gland cells themselves rather than to changes in the circulation is shown by the fact that stimulation of either the chorda tympani or the cervical sympathetics causes this increase in resistance in spite of the fact that the former stimulus produces an increase in the volume-flow of blood and the latter a decrease. Furthermore, there is neither resistance change nor secretion when the chorda tympani is stimulated subsequent to atropinization, although the volume-flow of blood is increased.

The results of certain investigators led us to expect a decrease in the resistance of the gland during secretion. Garmus (6), for instance, has found that the cells of the nictitating membrane gland of the frog are more than normally permeable to dyes when secreting under the influence of pilocarpin. Crozier (7) observed that during stimulation of the indicator containing tissue of the nudibranch Chromodoris a slimy secretion was expelled from the surface, and the rate of penetration of dichloracetic acid was greatly increased. And Lillie (8) states that "although the factors are complex, there seems to be little doubt that the transport of secretion across the cell boundary is associated with an increased permeability to water as well as to dissolved substances."

The difference between this generally accepted conception and our results may be accounted for by the fact that two differently functioning membranes are involved in the secretory process—those facing the lumen and those toward the basement membrane and adjacent cells. Thus it is possible that stimulation causes an increase in permeability on the lumen side but a decrease on the side toward the basement membrane. Inasmuch as the cell membrane toward the lumen has the smaller area (due to the wedge-shaped arrangement of the cells around the tubules and ductules) the result will be the observed increase in net resistance. If, in addition, there is a breakdown of intra-cellular substance into a larger number of osmotically active particles, water will be drawn in from the lymph and will carry with it the dissolved substances into the duct.

The two differently functioning membranes thus explain the properly directed secretory current as well as the resistance increase.

This conception of a differential change in membrane permeability may also be used to explain the passage of water from the osmotically more active blood into the osmotically less active saliva on the basis of electro-endosmose. Bernstein (9) among others has shown that if two solutions are separated by a semi-permeable membrane, it is possible for the water to flow from the more concentrated to the less concentrated against the osmotic pressure gradient. Due to the greater permeability of the membrane to certain ions than to others there is a difference in their concentrations on the two sides and consequently a potential difference between the two. And so the water molecules having acquired a positive charge by selective adsorption, or by contact, are caused to migrate from the positive to the negative side of the membrane. With a properly directed potential gradient the movement will therefore be against the osmotic pressure.

To explain the mechanism of resistance increase we might assume that in the resting condition the side of the cell toward the basement membrane is more or less permeable to both anions and cations with a consequently small or zero potential difference between the opposite surfaces of the cell membrane. If on stimulation the permeability to the anions within the cell decreases, there will be an increase in net resistance and a potential difference between the two sides of the membrane. Due to this potential gradient water will be drawn in from the lymph spaces and thus produce the secretion.

There are, of course, other possible factors which may play a contributory part at least in the secretory process and thus influence the resistance. It is, for instance, conceivable that the resistance variations are a function of cellular and intra-cellular acidity. It is well known that the conductivity of a protein salt solution depends on the acidity and is a minimum at the iso-electric point. Likewise, Osterhout (10) shows that the permeability of protoplasmic membranes varies with the concentration of H ions. With these facts in mind we set out to study the effects of mechanical asphyxia and administration of CO_2 on the response of the gland to the usual stimuli. The resulting increase in blood acidity threw no light on the problem, however, for the only observed effect of the increased blood acidity was a decrease in the resistance of the gland whereas glandular activity always caused both an increase in blood acidity and an increase in resistance.

Admitting that the complete answer to the problem may include other considerations, we will for the present hold to the tentative assumption that the one side of the cell decreases in permeability while the others increase or remain constant and that the secretion is effected by the

drawing through of water by osmosis or electroendosmose or by a combination of the two. The evidence in favor of the resistance variation being due to such a reversible change in membrane permeability is strengthened by the behavior of the gland during a series of stimuli with the duct alternately occluded and deoccluded. Figure 3 shows that after a prolonged stimulation with the duct occluded the resistance falls to below its pre-stimulus value. It is quite probable that this decrease is due to the injury suffered by certain of the cells in secreting against pressure for Osterhout (11) has found that an invariable consequence of cellular injury is a decrease in resistance. Furthermore, the usual result of decreased resistance resulting from injury is a decreased irritability. In this connection we have repeatedly found that when the gland is caused to secrete into an occluded duct, so that the resistance subsequently falls to below its pre-stimulus level, the next stimulus with the duct deoccluded produces less than the usual response,—the resistance increase, electrical deflection, and secretion all being smaller.

Influence of blood supply on resistance changes. If then the increase in resistance is due to a reversible change in the permeability of the membranes it seems reasonable to assume that the recovery of normal resistance is dependent upon an adequate blood supply. Stimulation of the chorda tympani for instance produces a rapid increase in resistance followed by a slower return to normal. Barcroft's determinations show that there is at the same time an increase in the oxygen consumption of the gland of from three to four-fold and an increased volume-flow of blood (12). If the recovery of normal resistance is dependent on blood supply, then stimuli which are accompanied by a smaller than normal increase in blood-flow should produce greater than normal increases in resistance because the change in the membranes would be able to proceed further before being effectively checked by the recovery process. This is shown to be the case by the unusually large increase in resistance resulting from stimuli with the duct or artery occluded, for in both instances the volume-flow of blood increases less than is usual on stimulation of the chorda tympani. The contrast between a temporary and a protracted deficiency in blood supply is likewise illustrated by these two types of stimuli. Following stimulation with the duct occluded the volume-flow of blood is greatly accelerated for some time, but after stimulation with the artery occluded there is no such increase. In the former case the resistance promptly recovers its normal value, while in the latter the recovery is usually much delayed. Here again resistance recovery seems to be conditioned by the blood supply.

The behavior of the gland as a result of stimulating the vago-sympathetic fibres during pilocarpin secretion gives another instance of the correspondence between reduced blood flow and increased resistance. The volume-flow of blood decreases and the resistance increases. This may

be accounted for as follows. During the time that the pilocarpin is effective in producing secretion it tends to keep the resistance at a higher level although this is constantly opposed by the recovery process. A state of equilibrium is attained resulting in a temporary constancy of resistance. When the volume-flow of blood is reduced by stimulation of the vago-sympathetics, the action of the pilocarpin in maintaining a higher level of resistance becomes more effective, the equilibrium is destroyed, and the resistance increases until a new equilibrium level is reached.

Stimulation of the vago-sympathetic fibres. It might be argued that if the result of a smaller blood supply is a greater increase in resistance, stimulation of the vago-sympathetics should produce a greater increase in resistance than a like stimulation of the chorda tympani. The reverse actually happens. The reason may lie in the fact that the two processes which we have suggested as combining to determine the size of the resistance change are better balanced from the start. The initial changes in permeability are probably much smaller than those resulting from chorda-tympani stimulation, as is evidenced by the smaller secretion; and the reduced blood supply is therefore adequate to hold the resistance rise to a low level. The effect of the reduced blood-flow is evident in the very much slower return of the resistance to its pre-stimulus value.

Increase in blood acidity. Our finding that the ever-present accompaniment of secretion is an increase in the acidity of the blood from the gland confirms Barcroft's (12) observation that the oxygen consumption and carbon dioxide output of the secreting gland are increased by a factor of three or four.

The greater increase in acidity that results from stimulation of the chorda tympani with the duct or artery occluded than from stimuli delivered when the duct or artery is not occluded is undoubtedly due to two effects of the smaller volume-flow of blood in the former cases. A given response of the gland results in the passage of a certain quantity of metabolites from the cells to the blood. A decreased blood-flow would therefore cause a greater concentration of acid metabolites and a correspondingly higher acidity. And, furthermore, the anaerobic conditions would probably cause a greater lactic acid formation. The increase in acidity when the carotid artery is occluded during pilocarpin secretion is also to be accounted for on the basis of the above two factors.

It is doubtful, however, if the differences in volume-flow of blood resulting from stimuli with the duct open or closed are sufficient completely to account for the greater blood acidity in the latter case. A plausible way of explaining the difference is to assume that the gland does more work in secreting against pressure and consequently has a higher rate of metabolism. Inasmuch as the blood-flow does not increase sufficiently to meet this greater demand for oxygen the result must be an increased acidity.

SUMMARY

Electrical resistance, electrical deflections and acidity of the venous blood of the submaxillary gland of the dog were photographically recorded.

A vacuum tube method to follow rapid changes in electrical resistance was developed, and the methods recently devised for following changes in acidity of the circulating blood were adapted to photographic registration with and without the use of the vacuum tube. The electrical deflections were recorded with the d'Arsonval galvanometer in conjunction with a vacuum tube.

The necessity of providing uniform conditions for the elicitation of uniform electrical deflection on stimulation of the chorda tympani was confirmed.

In marked contrast to the variability of the electrical deflections obtained under less stringent conditions was the uniformity of directional changes in electrical resistance.

Provided visible secretion was elicited an increase in electrical resistance invariably occurred.

The difference in behavior of the gland in these respects is probably accounted for by the fact that the electrical deflection is an algebraic sum of the potential differences of individual cells, whereas the net resistance is the arithmetic sum of the resistances of the several cells.

It is suggested that the changes in resistance may be accounted for by a constancy or decrease in resistance of the lumen side of the cell and an increase in resistance of the opposite side.

Stimulation of the cervical sympathetic fibres caused a smaller and less rapid increase in resistance than stimulation of the chorda tympani or the injection of pilocarpin.

With the duct occluded during stimulation of the chorda tympani the resistance increase was greater than normal, but subsequently fell below its pre-stimulation value. This may be due to cellular injury.

Retarded blood supply during stimulation likewise elecited a greater increase in resistance followed by a slower return to normal. This may be explained by the dependence of cell permeability and of recovery on blood supply—a retarded blood-flow permitting the resistance to reach a higher level before the establishment of equilibrium with the recovery processes.

Secretory activity of the gland was accompanied by increased acidity of the venous blood.

Secretory activity occurring along with decreased blood supply was accompanied by a greater increase in blood acidity.

Secretion with the duct occluded caused greater than normal increase in blood acidity.

Increased acidity of the blood produced by mechanical asphyxia resulted in decreased resistance of the gland.

No generalization relating resistance or acidity changes to electrical deflections seems permissible at present.

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THE VOLUME OF BLOOD IN THE HEART AND LUNGS

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When a healthy man passes from a condition of rest into heavy muscular activity the delivery of blood by the left ventricle increases enormously, an increase of from five to twenty-five liters a minute being an ordinary finding. It is known that a balance of constrictions and dilatations accompanies this increase in output, shunting blood toward the working muscles, and that the capillary bed in these muscles expands in area by the opening of closed vessels. Work is thus accompanied by an enlargement of the surface for diffusion of oxygen from the blood.

Since the delivery of the right ventricle must keep pace with the left, the increased blood flow of exercise plays also upon the pulmonary circuit and it is reasonable to expect adaptive vascular changes, particularly in the lung capillaries, which will result in providing a larger surface for diffusion of oxygen into the blood.

Many efforts have been made to measure vascular phenomena in the pulmonary circulation but the changes observed have not been impressive. Wiggers (1) has made a recent summary of this evidence. As an example of the issues which apply to muscular work one may take the question of adrenin effects. Though still a debated matter, it seems fair to say that the only result of adrenin injection into the pulmonary vessels is constriction. Liberation of adrenin into the blood stream is a usual accompaniment of muscular work but the action of the substance on the lung vessels would not appear favorable. Constriction of the pulmonary blood vessels by adrenin is not a conspicuous matter even with large dosage, and the conclusion is forced that the amounts of adrenin which reach the circulation in exercise have no effect on the lung vessels. The use of adrenin in studies on the lung circulation has but one substantial positive aspect, namely, the demonstration of a slight degree of sympathetic innervation.

The effect of other possible changes in blood composition is equally inconspicuous, though the quality of the investigations on this phase of the subject is not very convincing. Thus, it is not known whether blood reaching the lungs in the early stages of severe exercise, and relatively low in oxygen and high in carbon dioxide, affects the pulmonary vessels

directly. It is reactions in the capillaries which are of particular importance. Krogh (2) came to the conclusion that acidity caused by increasing the blood CO_2 had little effect as a dilator of systemic capillaries, and he was also unable to explain dilatation by oxygen lack. The effect on capillaries of acid metabolites of unknown character is suppositious, and finally none of the experiments on the subject deal specifically with the capillaries of the lung. Where an increase in the H-ion concentration of the blood has caused undoubted vascular dilatation it seems probable that the reaction, while undoubtedly independent of central influences has been largely in vessels well supplied with smooth muscle and not in capillaries (3). It is possible to consider that venous blood containing little oxygen and much carbon dioxide may cause a moderate dilatation of pulmonary arterioles—though any such reaction is as yet unidentified—but by analogy with the capillaries of other regions it seems improbable either that the lung capillaries dilate or that capillaries which have been closed become open. In a recent paper by Hall (4) describing the pulmonary capillaries in the rabbit and cat the conspicuous feature of these structures is their passivity. They were found to be exceedingly unreactive, taking care of the blood that comes to them in a singularly passive manner and never exhibiting disappearance, reappearance, or changes in calibre. This report coincides entirely with unpublished observations on the capillaries of the frog lung made several years ago in this laboratory and also with the apparent inability to detect the specialized cells of Rouget in the lung, assuming that they are the elements responsible for capillary reactions.

Spehl and Desguin (5), employing rabbits and using a closed chest technique, measured the blood in the lungs and in the entire body. Their methods, which are similar to those used by Spehl (6) and by Heger and Spehl (7), consist in an abrupt ligation of all the vessels entering and leaving the heart, this ligation taking place at the end of inspiration or expiration. In Brussels, at sea level, they found in eight animals that the pulmonary blood volume measured at the end of inspiration was 1/11.8 of the total blood volume. Eight animals examined similarly in the Col d'Olen laboratory at an altitude of 3000 meters had a pulmonary blood volume averaging 1/9.4 of the total blood volume. Most of the rabbits were exposed to the high altitude not longer than four days. One remained for ten days. Observations on two animals at sea level gave an average pulmonary blood volume at the end of expiration of 1/18.8 of the total blood, and on two animals similarly measured in the Col d'Olen Laboratory gave an average value of 1/12.1. These observations are not numerous. They are not correlated with any data on blood oxygen, blood carbon dioxide, nor character of respiration, and must simply stand as unverified testimony of the possible fact that at high

altitudes there is an enlargement of the pulmonary vascular bed which occurs in the course of a few days and is of measurable extent.

On the side of nervous control the pulmonary vessels are equally unreactive. Sympathetic fibres are present, demonstrated both anatomically and by use of adrenin. Attempts at nerve stimulation have resulted in reports of no effect and of both rises and falls of pulmonary arterial pressure. The only evidence which applies directly to the issue we have raised—namely, the question of enlarged accommodation for the increased pulmonary blood flow of exercise—is that given by Sharpey-Schafer (8) who found that excitation of the depressor nerve in the rabbit causes a variable and never great fall in pulmonary arterial pressure accompany-

TABLE 1

Volume of blood in the lungs in relation to total blood volume. Observations on rabbits and dogs killed at the end of inspiration and expiration. Closed chest preparations

OBSERVER	NUMBER OF ANIMALS	PULMONARY BLOOD VOLUME IN RELATION TO TOTAL BLOOD VOLUME	
		End of inspiration	End of expiration
Rabbits			
Heger and Spehl (7)	4	1/12.5	
	3		1/17
Spehl and Desguin (5)	16	1/10.7	
	4		1/15.3
Spehl (6)	1	1/12.3	
	1		1/16.6
Dogs			
Plumier (9)	4	1/9	
	6		1/10.66

ing the usual fall in systemic pressure. In the absence of more thorough control of blood flow it is impossible to consider that these experiments give any certainty that pulmonary vascular relaxation is an ordinary depressor nerve effect.

The work of Spehl and Desguin (5), to which reference has been made, indicates that as a passive effect of the respiratory movements the amount of blood in the lungs may be altered. Results upon this point in closed-chest animals are shown in table 1. It is apparent that the inflated lungs contain more blood, though not much more, than the deflated. How great this increase can be if respiration is vigorous is not known. It is

however, evident that associated with the movements of breathing and possibly independent of vascular reactions, there is provision for increasing pulmonary blood.

Kuno (10), working on dogs and using a heart-lung preparation in which it was possible to alter cardiac inflow and calculating total blood volume as 7 per cent of the body weight, obtained the results shown in table 2. Artificial respiration was necessary in these experiments and the chest was open. The figures are, however, interesting since they show what a significant augmentation of pulmonary blood volume accompanies increased cardiac inflow.

Stewart (11) made indirect determinations of pulmonary blood volume in dogs, using the formula: $V = Q\frac{60}{T}$ in which V is the minute volume of the heart in cubic centimeters, Q the average quantity of blood in the lungs, and T the pulmonary circulation time. T and V were measured, and Q was calculated. This method permitted multiple determinations

TABLE 2

Volume of blood in the lungs in relation to cardiac output in heart-lung preparations on dogs. Assembled from Kuno (10)

WEIGHT	CARDIAC OUTPUT	BLOOD VOLUME		PULMONARY BLOOD VOLUME / TOTAL BLOOD VOLUME
		Pulmonary	Total	
kgm.	*cc.*	*cc.*	*cc.*	
7.0	680	43.1	490	1/11.3
8.1	800	58.98	567	1/9.4
4.6	1350	40.75	322	1/7.8
4.25	1320	44.8	296	1/6.6
5.1	1900	69.41	357	1/5.1

on the same animal, but it was discontinuous, and since each measurement involved the injection of either dye or salt solution, uniform conditions of observation could not be maintained. Furthermore, the complete validity of the methods used to determine V and T is open to criticism. In the same paper Stewart reports some direct experiments on dogs "to determine the extreme range of the amounts of blood contained in the lungs under different conditions." He summarizes his results as follows:

When the outflow through the aorta was completely blocked, the inflow into the right heart being unobstructed or at least the inferior cava open, the lungs contained 22 per cent of the total blood in one animal and the heart and lungs together 27 and 30 per cent in two animals. When the block on the right side was complete, or at least the pulmonary artery was entirely blocked, while the outflow from the left side of the heart was either entirely free or only partially obstructed, the lungs contained 6, 9, 7, 3.5 and 5 per cent of the total blood in five animals. When both sides of the heart were completely obstructed simultaneously the lungs contained 21 per cent and 18.6 per cent in two animals. In an animal bled to death the lungs contained

3 per cent of the total blood. In an animal killed by passing a strong current through the heart the lungs contained 9 per cent, and the lungs and heart together 25 per cent of the total blood.

The dogs used in these experiments were anesthetized and were breathing naturally with chests which had been undisturbed. The obstructions noted were accomplished by injecting melted wax through jugular and carotid catheters. Stewart points out the obvious difficulty of making this technique precise. His figures are, however, valuable in their demonstration of the possible capacities of the heart and lungs.

In summary, the literature on pulmonary blood volume contains no evidence that the pulmonary vascular bed is altered significantly through chemical changes in the blood. The possibility of reflex effects through the depressor nerves exists, and if such changes occur they are towards dilatation. Passive changes in the size of the bed occur in respiration, increase accompanying inspiration. A much more significant augmentation accompanies increased cardiac inflow. As a result of blocking outflow from the left ventricle the lungs may contain 22 per cent of the total blood volume and the heart and lungs together 30 per cent. When the pulmonary artery was blocked the lung blood fell to as low a figure as 3.5 per cent of the total volume.

All of these observations lack continuity. They are the results of single experiments on different animals or widely separated observations on the same animals.

Our experiments have employed a technique which permits continuous and simultaneous measurement of *a*, cardio-pulmonary blood volume, *b*, cardiac output, *c*, systemic blood pressure. The cats used have had closed chests, the lungs being inflated and filling the thoracic cage, and have been under artificial respiration. Cardio-pulmonary blood volumes have been measured under the following sets of conditions:

1. Cardiac inflow being constant, ventilating gas mixture altered to produce:
   a. Increase in blood carbon dioxide.
   b. Decrease in blood oxygen.
2. Ventilating gas mixture being constant:
   a. Blood flow, rate and depth of artificial respiration varied.
3. Ventilation being constant:
   a. Occlusion of left branch of the pulmonary artery.
   b. Occlusion of right branch of the pulmonary artery.
4. Ventilation being constant:
   a. Partial to complete occlusion of the pulmonary veins.

TECHNIQUE. A. *Preparation of animal.* The cat is lightly anesthetized with ether in order to cannulate the femoral vein, and then is given 5 per cent barbital sodium intravenously. The injection is begun as the

animal is recovering from ether, and 20 to 28 cc. give an extraordinarily good anesthesia with great promptitude. The injection requires but a few minutes and is not given in accordance with body weight but by direct observation of the minimum amount required to induce complete surgical anesthesia. When barbital sodium works unfavorably the respiration is the source of danger, but given in this manner the dose can be regulated so perfectly and supplemented so readily that little danger need be anticipated. After anesthesia is complete the animal is curarized in order to prevent convulsive movement when aortic blood flow is interrupted in shifting from the natural to the artificial systemic circuit. A cat anesthetized in this manner is placed upon a warm animal board, and the heart is exposed and the chest closed by means of the pericardium as described in a former paper (12). It has been found very important to use a gas mixture for artificial respiration which contains not less than 5 per cent carbon dioxide. Prolonged experience indicates 7.5 per cent to be very nearly the correct figure.

B. *Dissection and adjustment of the artificial peripheral system.* Following complete closure of the chest the lungs are brought out so as to fill the thorax, this condition being readily ascertained by direct view through the transparent pericardium. The blood of a second cat, rendered incoagulable by the addition of heparin, is now placed in the artificial peripheral system which is best described by reference to figure 1.

The heart, *1*, beats freely on the surface of the pericardial sling, *2*. The right auricular appendage is lifted with forceps and a fair section of its tip clamped with a soft-closing smooth-jawed hemostat. The free margin of the appendage is opened and the cannula, *25*, is tied into it and connected with the inflow tube, *24*. The cannula and the inflow line, *24*, are now filled with blood from the reservoir, *17*, all air being expelled through the short upright arm on cannula *25*. When this has been accomplished the clamp, *23*, which is an adjustable machinist's caliper square is closed, and the hemostat on the auricular appendage is removed. There is now a slight mixing of the blood in the right auricle and in the cannula, but the latter contains enough heparin to render coagulation impossible. The actual length of the inflow tube is short, and by careful up-and-down adjustment of clamp *23*, cannula *25* is made to enter the auricular appendage without possibility of obstructing inflow due to kinking off. In the Knowlton-Starling heart-lung method and in such slight modifications as have appeared, blood inflow has been accomplished by cannulation of the superior vena cava. If this is done in the closed-chest preparation used by us, the position of the heart has to be distorted and the actual intrapericardial length of the superior cava is so short as to make the insertion of the cannula quite difficult. We have, consequently, taken the liberty of letting the blood into the right side of the heart through the

tip of the appendage and, so far as we can see, this new procedure does not disturb cardiac function in the least.

These adjustments have been made under artificial respiration, the pump described in a previous paper (13) being employed. The superior vena cava, *5*, is now tied and the inferior vena cava, *6*, occluded by raising it upon the ligature previously placed around it. Under these circumstances all blood flow into the heart is cut off. The aorta is at

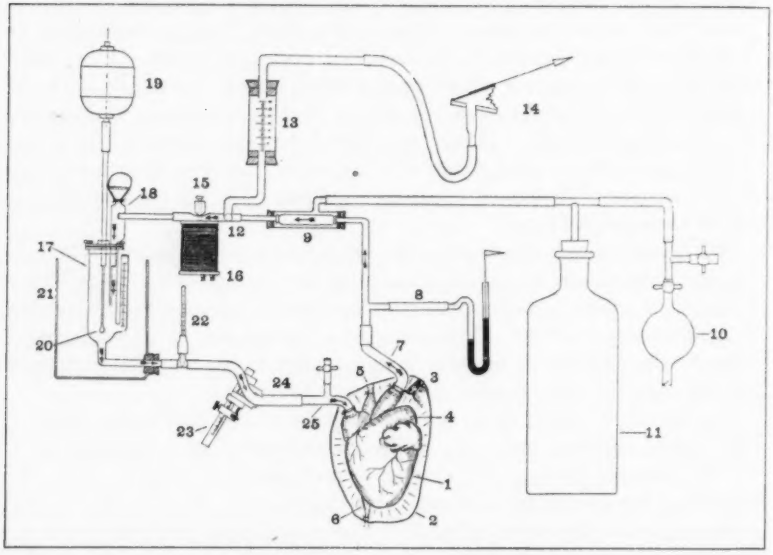


Fig. 1. Arrangement of peripheral circuit in isolated heart and lungs. Diagrammatic. Different parts not on same scale. *1*, heart; *2*, pericardium supporting heart and stitched to margins of thoracic opening; *3*, aorta; *4*, pulmonary artery; *5*, superior vena cava; *6*, inferior vena cava; 7, aortic cannula; *8*, blood pressure line to mercury manometer; *9*, capillary resistance; *10*, bulb for raising pressure; *11*, pressure reservoir playing upon *9*; *12*, T-tube leading to flow-measuring device; *13*, flow measuring reservoir; *14*, Brodie bellows recorder; *15*, soft iron hammer resting on rubber tube; *16*, magnet for drawing down iron hammer and closing blood circuit to venous reservoir; *17*, venous reservoir; *18*, inflow into venous reservoir; *19*, small motor operating stirrer; *20*, stirrer; *21*, water-bath, electrically controlled; *22*, thermometer in inflow line; *23*, clamp; *24*, inflow line; *25*, cannula in right auricular appendage.

once incised and the outflow cannula, *7*, thrust into it and tied in by the ligature previously placed around the vessel. Just as soon as this tie is secure, the inferior vena cava is released and natural cardiac inflow from this vessel is resumed. Blood flows from the lungs into the left side of the heart, and is driven out through the aorta into the peripheral system. This blood is, of course, coagulable, and in order to care for it heparin

solution in small amounts is injected slowly into the rubber connection leading away from aortic cannula, *7*. As soon as the natural blood flow ceases, due to exsanguination of the cat, the inferior vena cava is tied and the clamp, *23*, opened. The heart now receives blood from the inflow side of the artificial system and after passage through the lungs this blood is returned again to the periphery via the aorta. *It is a simple heart-lung preparation with the lungs inflated and enclosed in the thorax.* The outflow cannula, owing to the distensibility of the aorta, can be given an inside diameter equal to that of the aorta, and the inflow cannula inserted in the auricular appendage can be made amply large to care for inflow increases. Artificial respiration must, of course, be used since the animal is dead except for the heart and lungs. It is possible in open chest preparations to adjust respiration so that the lungs are held in a normal state of inflation and do not reach the condition of complete deflation so often seen in such experiments. We have, however, felt that the lungs can be kept in more normal condition by closing them in the chest and excluding air than in any other manner, and believe that in experiments requiring any length of time closure technique is advisable.

C. *Blood-flow measurement.* Many different methods for accomplishing this were considered and actually tried. The facts that the arrangement employed must record constantly and must show large variations in flow caused us to discard a variety of schemes. The technique finally used is an adaptation of a method described by Livingston (14) and has proved highly efficient. At *12*, in figure 1, a T-tube is inserted in the line. The free limb of this T extends horizontally out from the regular line of flow. It is connected by means of a U-tube and rubber stopper with reservoir *13*. This reservoir is a section of a 250 cc. graduate. It is closed at the top by a rubber stopper carrying a glass tube. Just beyond T-tube *12* there is a short flexible rubber connection upon which rests a soft iron cut-off, *15*. This cut-off, held across the rubber tubing by means of upright bars at both ends, is drawn down whenever magnet *16* is activated, and the natural flow line to the venous reservoir is occluded just so long as magnetization lasts. The magnet is activated and cut off for regular brief periods by means of a rotating circuit breaker which operates with extreme precision. The result of this arrangement is that, during definite and constant periods, blood is diverted from the regular line of flow out through the T, and rises during the period of occlusion in reservoir, *13*. The degree of this rise is recorded on a kymograph by means of a calibrated Brodie bellows, *14*, operated by air displacement. When the magnetic hammer, *15*, is down, blood flows into *13* and the Brodie bellows rises. As soon as the hammer is released flow in the natural direction occurs once more and the blood pushed out into *13* returns into the circulation. The height to which this diverted fluid rises in reservoir

13 depends on the vigor of cardiac discharge. If the heart is delivering a large minute volume, 350 to 400 cc. per minute, and the bottom of reservoir *13* is just above the level of tube *12*, there will be a certain amount of storage in the reservoir. That is, for a time more blood will be pumped into the reservoir during occlusion periods than runs out during the longer open periods. In a few moments, however, equilibrium is reached and after this, though the level in *13* may be as much as 6 cm. above line *12*, it is sustained there, increasing slightly at each closure of the magnetic cut-off and decreasing at each opening. The type of record obtained is shown, figures 4, 10, 12 and 14.

D. *Blood volume measurements.* In a preparation such as the one described, the circulating blood is divisible into two parts, one within the heart and lungs and the second within the artificial system of glass and rubber. The amount in this second system can be read constantly by recording simultaneously the heights of the columns of blood in reservoir *17* and in the flow recording chamber *13*. If, for example, the pulmonary veins are obstructed so that the output of the left ventricle decreases, blood will accumulate in the lungs and in the right side of the heart. This accumulation must be at the expense of the outside system and will be measured promptly and exactly by the fall in reservoir *17* and in the flow recording chamber *13*. Furthermore, if at the close of the experiment the total blood volume is measured directly or if Congo red is placed in the circuit and, after allowing a few minutes for mixing, the blood volume is calculated in the usual manner, a figure will be obtained which represents the total volume of blood in the peripheral artificial system plus that in the heart and lungs. If the total volume of blood is known one can obtain a figure for the blood in the heart and lungs by subtracting the volume of blood in the peripheral system. In actual experiments an observer was constantly employed in making readings of the blood height in reservoir *17* and in chamber *13*. These readings, recorded at definite times, represent figures which on subtraction from the total blood volume gave the heart and lung volume at the moment. The method is simple and, since it has no effect on the integrity of the preparation, can be applied continuously through fairly long experiments. There is one factor connected with it which is absolutely essential for success. No blood must be lost from the system during the experiment. There are three ways in which blood may escape.

1. *By evaporation.* If the air or gas mixture used to ventilate the animal is dry (15), evaporation may cause a certain degree of blood concentration. This source of loss is easily prevented by thorough saturation with water vapor of the air or gas used for ventilation.

2. *Through the development of pulmonary edema.* Anrep and Cannan (15) have shown that in the heart-lung preparation the blood carbon

dioxide falls markedly, and there is a shift in reaction toward the alkaline side. This change may be a factor in the production of progressive pulmonary edema. By using air containing carbon dioxide in amounts between 5 and 10 per cent, carbon dioxide loss from the blood can be prevented. Attention has already been called to the use of carbon dioxide in the ventilating gas. Our experiments have not been marred by the occurrence of edema except as a result of pressure increases in the pulmonary vessels or radical changes in blood gas content, and these changes have constituted definite experiments in themselves.

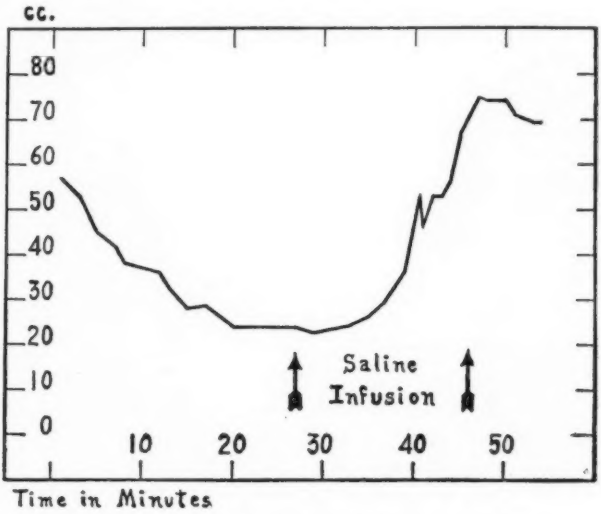
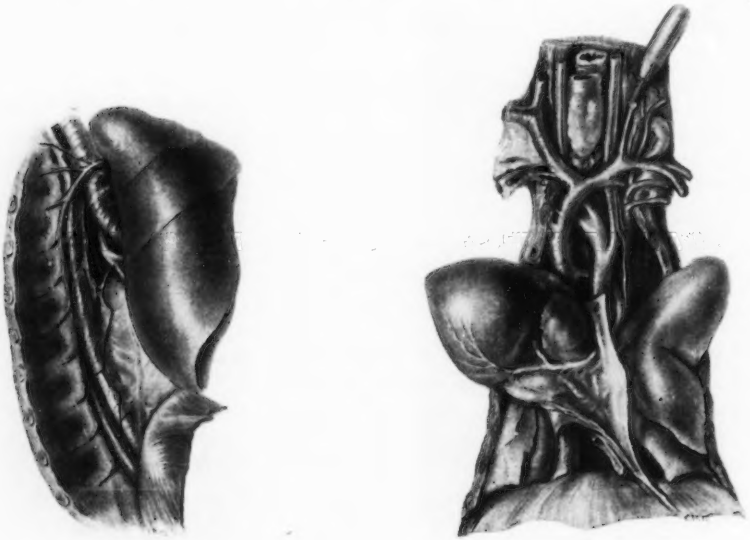


Fig. 2. Curve showing the fall in volume of blood in the venous reservoir, *17*, during 27 minutes, and the gain in volume which occurred on infusing a very large amount of salt solution (840 cc.) into the femoral vein. Ordinates, cubic centimeters of blood in reservoir. Abscissae, minutes. Saline infusion given between arrows.

3. *Through anastomoses with the general circulation of the animal.* All who have had experience with the heart-lung circulation have observed occasions in which the system lost blood. If the volume of blood makes no difference such losses are made up by pouring more blood into the venous reservoir. In our experiments any such method of merely keeping things going is not permissible, since a determination of total blood volume at the close of the experiment must be related to a series of readings of the volume in the peripheral system taken during the experiment and it is thus essential that the total volume remain constant. A typical

instance of blood loss is shown in figure 2. In this case, during twenty minutes, the blood in the venous reservoir (*17*) fell from 57 cc. to 44 cc. The level then became constant and remained so until, under the influence a large saline infusion into one femoral vein, the volume was brought back to the original level and pushed up to 75 cc. In normal life the cat in question had an anomalous communication between the aorta and pulmonary veins (fig. 3, A). When placed in the heart-lung preparation this



A B

Fig. 3. A. The anastomosis causing the loss of blood seen in figure 2. A small vessel entering one of the pulmonary veins gives off branches which penetrate the esophagus and eventually ends in the aorta.

B. In this case the coronary sinus, instead of entering the right auricle directly, ends in a comparatively long vessel which passes under the aorta and enters the superior vena cava. In the actual experiment the vena cava was tied between the entrance of this vessel and the heart. As a result all of the coronary blood was pumped out into the body of the cat and the experiment terminated abruptly.

anastomosis carried blood in the reverse direction, there being a very slight pressure in the pulmonary veins and no pressure in the aorta. After a certain amount of leakage this pathway ceased to operate, but when the veins of the cat were overfilled with salt solution, some of which in the absence of aortic pressure passed back through the capillaries into the aorta, flow towards the pulmonary veins took place and the circuit was then replenished from the vessels of the dead part of the animal.

A second and rarer anomaly is seen in figure 3, B. Here all of the coronary blood was pumped out into the superior vena cava through an extension of the coronary sinus. Nothing could prevent such a blood loss except the fortunate accident of placing the caval ligature peripheral to the entrance of the anomalous coronary vessel. Another and unimportant source of loss is found in extremely small vessels leaving the circulation of the heart or passing out from the great vessels at the base of the heart or in the pulmonary system and running along the vagus and phrenic nerves and into the mediastinal fat. Hair-like vessels of this general type can usually be found by means of careful injections. They are of little moment and are always taken care of by the blockage manoeuvre to be described. The bronchial arteries are in the same category.

If the heart-lung preparation is working properly, that is, if rate and output and general appearance of the heart are uniform and excellent and if the peripheral part of the system contains a graduated reservoir, readings of the blood height in this reservoir will soon show whether or not blood is being lost into the vessels. In about half the animals used, leakage does not take place or is so slight as to escape measurement. In our experience, barring such a situation as that seen in figure 3, B, loss of blood can always be prevented by passing a long cannula up the aorta and injecting through this cannula melted vaseline to which enough ordinary embedding paraffine has been added to cause a waxy solidification at body temperature.

E. *Measurement of heart volume.* Any value which our studies possess turns upon the degree to which they indicate capacity changes in the pulmonary circuit. We have not been able to subtract cardiac blood from lung blood in a decisive manner and have, therefore, been compelled to present our data in terms of combined volume. It has, however, been possible on using such a manoeuvre as increasing the blood CO_2 to exclude ventricular cardiac dilatation from the results. This has been accomplished by using a capsule cardiometer of the general type described by Yandell Henderson (16). In our hands it has required a great deal of patience and care to make this instrument record with approximate accuracy and even when applied under the best possible circumstances it gives actual curves for systolic discharge, which, when calibrated for volume, invariably fall below cardiac output as measured accurately at the same time by the device used in our experiments. Failure of the cardiometer to follow systolic discharge is not of moment in our requirements. We have wished a measure of increase and decrease in ventricular size, and there is no reason why this desire has not been realized. The cardiometer used consists of a light glass funnel with hemispherical bottom —a thistle tube without the lip—across the mouth of which are tied two thicknesses of condom membrane. A hole is made through both layers

of membrane with a hot bar of metal, the diameter being entirely a matter of judgment and depending on the size of the heart. In applying the cardiometer it is dipped in melted vaseline and a silk thread tied to the apex of the left ventricle is passed through the hole and out through the stem of the funnel. The cardiometer is slipped over the heart so that the ventricles are well within it, the extreme base of the right ventricle being lost to some extent since it is impossible to push the cardiometer membrane all the way up to the pulmonic valve. The silk ligature extending through the funnel is now threaded on a straight needle and passed through the wall of the rubber tube connecting the cardiometer with the Brodie bellows used for recording. The ligature acts as a most efficient "stay" in holding the heart in a uniform position throughout the period of experiment.

A ventricular cardiometer of this sort, even when attached to the most sensitive bellows recorder, fails to follow the quick movements of the cat heart but does react so as to give a satisfactory picture of slow changes in the total volume of the ventricles. The fact that the auricles are not included in the cardiometer introduces an error, which is probably never greater than 2 cc. That is, when the entire heart dilates the ventricular part of this dilatation is measured. The auricular increase in size is lost and is probably not more than 2 cc. In our figures this would appear as lung blood.

A complete protocol together with the methods of calculating is given for the first experiment cited. In other cases the figures alone are sufficient. The experiments are apparently complex but with experience and team-work the amount of time required has fallen within very reasonable limits. For example, in the first experiment from the beginning of anesthesia to the completion of the heart-lung preparation, 65 minutes were required. The observations in this case were finished in 49 minutes. The extreme duration, figuring from the beginning of anesthesia in any of the experiments cited was 142 minutes and the shortest duration 82 minutes. One cannot exaggerate the importance of perfect operative conditions in all such experiments. Over-ventilation, chilling, and clumsy handling have been responsible for more than a little of the suspicion which has grown up about mammalian experiments of radical type.

Experiment I. December 10, 1925. Cat, weight 3.7 kgm. Figures 4 and 5.
Determination of the volume of blood in the heart and lungs with:

1. Change of ventilating gas from 5.00 per cent CO_2 + 95 per cent O_2 to 20.31 per cent CO_2 + 79. 69 per cent O_2.
2. Change of rate of cardiac inflow.

10:15 a.m. Brief ether anesthesia for injection of 20 cc. 5 per cent barbital sodium into femoral vein.

10:25 Artificial ventilation with 5 per cent CO_2 + 95 per cent O_2 at rate of 32.5 per minute, and depth of 13 cc. per breath, giving 422.5 cc. per minute. Volume and rate unchanged throughout the experiment.
10:26 Curare 2 cc. 2 per cent solution intravenously.
11:20 Heart-lung preparation complete with wax injection.
11:25 Sodium bicarbonate 0.125 gram added to blood.
Systemic blood pressure held at 96 mm. throughout.

Observations

TIME	REMARKS	RESERVOIRS		FLOW	T	VOL. H AND L	CALCULATIONS	
		No. 17	No. 13				Cardiometer correction	Vol. H. and L. (Heart constant)
				cc./min.		*cc.*	*cc.*	*cc.*
11:29	Ventilating gas, CO_2 5.00 per cent, O_2 95 per cent	73.5	35	294	37°	29.5	0	29.5
11:29½		73.5	35			29.5	0	29.5
11:32		73.5	36			28.5	0	28.5
11:34		74.0	37	280		29.5	0	29.5
11:35	Alveolar air no. 1, CO_2 6.25 per cent							
11:36	Blood gas analysis 1, CO_2 46.36 vol. per cent, O_2 17.93 vol. per cent							
11:37		75.0	33	267		27.5	0.5	27.0
11:37½		75.0	32			28.5	0.5	28.0
11:38	Ventilating gas shift to CO_2 20.31 per cent, O_2 79.69 per cent							
11:40				253				
11:41		75.5	32			30.5	2.0	28.5
11:43		76.0	32	253		32.5	2.0	30.5
11:43½	Blood gas analysis 2, CO_2 65.19 vol. per cent, O_2 16.47 vol. per cent							
11:44		78.0	31	240	37°	31.5	2.0	29.5
11:45		78.0	31			31.5	2.0	29.5
11:45½		78.0	31	240		31.5	2.0	29.5
11:46	Alveolar air no. 2, CO_2 19.43 per cent							
11:48½		78.0	31	245		31.5	2.0	29.5
11:49	Ventilating gas return to CO_2 5.00 per cent O_2 95 cent							
11:49½		77.5	32			29.5	1.5	28.0
11:50½		77.5	32			29.5	1.5	28.0
11:51		77.5	32			29.5	0.5	29.0
11:52		77.5	33	267	36°	28.5	0.5	28.0

TIME	REMARKS	RESERVOIRS		FLOW	T	VOL. H AND L	CALCULATIONS	
		No. 17	No. 13				Cardiometer correction	Vol. H and L (Heart constant)
				cc./min.		*cc.*	*cc.*	*cc.*
11:52½	Blood gas analysis 3, CO_2 50.22 vol. per cent O_2 18.78 vol. per cent							
11:54½		80.0	32	256		20.5	0.5	29.0
11:55		80.0	33			30.5	0.5	30.0
11:59	Alveolar air no. 3, CO_2 5.65 per cent			267				
11:59½		80.5	33			32.5	1.0	31.5
12:00		80.5	33			32.5	1.0	31.5
12:00¼	Inflow opened							
12:00½		86.5	49	534		36.5	2.0	34.5
12:01½		87.0	50			38.5	2.0	36.5
12:01¾		87.0	51			37.5	2.0	35.5
12:02½		88.0	54			38.5	2.0	36.5
12:03½		88.0	55	534		37.5	2.0	35.5
12:04		88.0	55			37.5	2.0	33.5
12:04½	Inflow slowly closed	88.0	55			37.5	2.0	35.5
12:05¼		88.0	53			39.5	2.0	37.5
12:05½		86.5	47			39.5	2.0	37.5
12:06		78.0	26			26.5	0.5	26.0
12:06½		77.5	26	117		24.5	0.5	24.0
12:07½	Inflow opened							
12:08		83.0	40			32.5	1.5	31.0
12:08½		85.0	43	405	37°	37.5	1.5	36.0
12:09	Inflow completely closed							
	Terminal reading	73.0	21					

Autopsy notes. Slight residual pneumothorax. A few scattered areas of atelectasis in both lungs, particularly in the dependent portions of the lower lobes. No edema. Except in atelectatic areas the lungs expand and deflate with the moderate pressure artificial ventilation employed.

Weight of organs: Heart, 18.08 grams; lungs, 37.7 grams.

Blood recovered from apparatus: Reservoir 17 and inflow 120 cc.; reservoir 13 and attached tubes 30 cc.

Determination of amount of blood in heart and lungs by hemoglobin estimation: Heart: 3.0 cc., lungs: 8.5 cc.

This low volume in the heart and lungs at the close of the experiment is due to the fact that in this and other cases the inflow line 24, figure 1, has been closed in order to terminate the experiment and this results in pumping a maximum amount of blood into the peripheral system and out of the heart and lungs.

Calculations:

	cc.
At terminal reading of 73, blood in reservoir 17	92
Constant volume of tubing from reservoir to heart	28
	120
(Actually recovered)	120
At terminal reading of 21, blood in reservoir 13	3
Constant volume of system of tubing	27
	30
(Actually recovered)	30

	cc.
Reservoir #17	92
Reservoir #13	3
Constant volume all tubing	55
Blood in heart	3
Blood in lungs	8.5
Total blood at finish	161.5
Removed during experiment for analysis	30.0
Total blood at start	191.5

Subtracting the volume contained in the unvariable systems of tubing from the total blood at the start (191.5 − 55.0 = 136.5) gives 136.5 cc. as "working total" at the start. This volume is distributed among the three variable portions of the entire system, the two reservoirs and the heart and lungs. This "working total" is employed in the calculations listed in the table of observations, and is reduced by 10 cc. by each sample of blood taken for analysis.

Example:

11:29 Reservoir no. 17: Reading of 73.5 in arbitrary units is known by calibration to mean 90 cc.

Reservoir no. 13: Reading of 35 in same manner is known to indicate 17 cc.

90.0 + 17.0 = 107.0 cc. in the two reservoirs.

Subtracting this volume from the "working total" (136.5 − 107.0 = 29.5) gives 29.5 cc. as the volume of blood in the heart and lungs at this time.

Cardiometer correction. Correction may now be made for the dilatation of the heart which occurs during the course of the experiment. This is determined by converting the distance from the base line to the cardiometer tracing on the drum to cubic centimeters. The calculated increment, indicating a corresponding increase in the volume, or dilatation of the heart, is then to be subtracted from the above obtained volume of blood in the heart and lungs, giving a figure which represents the volume of blood in the heart and lungs with the heart volume considered constant. Changes in this amount may therefore be considered to represent changes in the volume of the pulmonary circuit.

Example:

TIME	DISTANCE FROM BASE	INCREMENT	INCREMENT	USED AS NEAREST 0.5 CC. FOR CALCULATION
	mm.	*mm.*	*cc.*	
11:29	89.2	0	0	0
11:34	90.0	0.8	0.14	0
11:37	91.3	2.1	0.38	0.5
11:41	101.0	11.8	2.14	2.0

Fig. 4. Sections of the kymographic record obtained in experiment 1. A, tracing of Brodie bellows recording blood flow in the aorta; B, traced by a second Brodie bellows and recording the volume of the ventricles through a capsule cardiometer; C, tracing of blood pressure by mercury manometer; D, base-line for blood pressure; E, time in 6-second intervals.

Figure 4 is a section of the kymographic record made during this experiment. *A* is blood flow; *B*, heart volume; and *C*, blood pressure. At *1*, the gas with which the animal was being ventilated was changed from 5 per cent CO_2 and 95 per cent O_2 to 20.31 per cent CO_2 and 79.69 per cent O_2. This point is marked with an arrow on figure 5. Reading from left to right, the second slip of tracing shows the condition two minutes later. The heart is somewhat slowed, and has begun to dilate but shows no change in delivery. At *2* the ventilating gas was returned to the original mixture. Before this time there has been very slight falling off in aortic output and the cardiac dilatation has slowly become greater. On returning to the 5 per cent CO_2 mixture there is a prompt recovery from dilatation. At *3* the cardiac inflow which has been kept constant was increased. There is a prompt rise in aortic output from 267 to 543 cc. per minute. This occurs rapidly and is not accompanied by very much cardiac

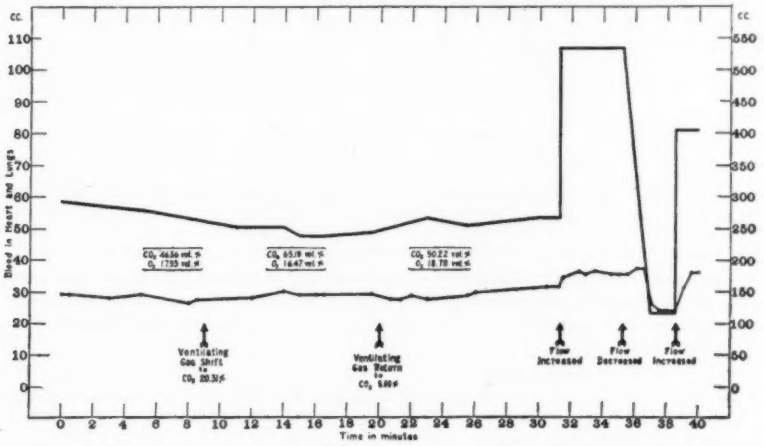


Fig. 5. Chart of experiment 1. Effect on pulmonary blood volume of increasing blood CO_2 and blood flow. Upper line, aortic blood flow. Lower line, volume of blood in heart and lungs corrected to exclude increases and decreases of volume due to cardiac changes in size. Each dot on this line indicates a separate set of readings giving a determination of cardio-pulmonary blood volume. On the left, ordinates for volume of blood in the heart and lungs; on the right, ordinates for aortic blood flow. Abscissae, time in minutes.

dilatation. The dilatation becomes slightly less during the period up to *4* when cardiac inflow was decreased, altering the aortic flow from 534 to 117 cc. per minute. At *5* inflow was again increased, and the aortic output rose promptly to 405 cc. per minute.

Figure 5 is a chart giving the essential details of the experiment. The solid upper line is the aortic blood flow, the output of the left ventricle minus the undetermined fraction of blood in the coronary circuit. The lower line is the volume of blood in the heart and lungs corrected for cardiac dilatation. That is, upward increments mean blood pooled in the lungs, not blood added to the heart and lungs by cardiac dilatation as noted in the discussion of figure 4. The three sets of figures between the two lines of the chart are the results of analyses of blood taken from the peripheral system just in front of the capillary resistance (*9*, fig. 1). It will be observed that

when the blood is increased in CO_2 from 43.36 vol. per cent to 65.19 vol. per cent, there is practically no change in the volume of lung blood. A slight suggestion of rise is perhaps present but it is too little for comment.[1] On the other hand, increase and decrease in blood flow change the pulmonary blood volume, instantly and markedly.

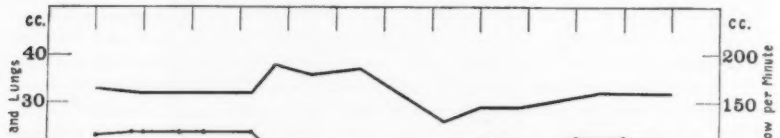


Fig. 6. Effect on pulmonary blood volume of giving adrenin and increasing blood CO_2. The system of charting is identical with figure 5.

[1] In several experiments the H-ion concentration of the blood or plasma has been followed. In the above experiment the values obtained for the plasma in the three specimens on which oxygen and carbon dioxide figures are given were as follows:

BLOOD GASES	pH GASOMETRIC	pH COLORIMETRIC
1. CO_2 = 46.36 vol. per cent..... O_2 = 17.93 vol. per cent.....	7.28	7.3
2. CO_2 = 65.19 vol. per cent..... O_2 = 16.47 vol. per cent.....	6.90	7.0
3. CO_2 = 50.22 vol. per cent..... O_2 = 18.73 vol. per cent.....	7.35	7.35

It is sufficiently obvious that the blood has become quite acid during the period of high CO_2 administration. The technique for obtaining these values was as follows:

Collection of alveolar air. The tracheal cannula, 17, possesses a round opening opposite the single tube which is tied in the trachea. This opening is covered with rubber dam. A ureteral catheter is passed through a pin hole in this dam down the trachea until positive bronchial obstruction is met. It is then withdrawn slightly and is connected with a 100 cc. glass syringe. A few centimeters of air are drawn into the syringe and expelled, and then a 50 cc. sample is obtained. The operator withdraws this at a uniform rate, endeavoring not to get ahead of the artificial respiration.

Hydrogen ion concentration of the plasma. The hydrogen ion concentration of the plasma was measured colorimetrically and gasometrically.

In the absence of a correction factor for cat plasma it was thought advisable

Experiment II. December 7, 1925. Cat, weight 3.3 kgm. Figure 6.
Determination of the volume of blood in the heart and lungs with:

1. Introduction of adrenin and sodium bicarbonate.
2. Change of ventilating gas from 8.5 per cent CO_2, 40 per cent O_2, and 51.5 per cent N to 20.43 per cent CO_2, 40 per cent O_2, and 39.6 per cent N.

The details of the experiment are shown in figure 6. After an initial period of a little over 6 minutes, during which a sample of blood shows CO_2 40.8 vol. per cent, 0.125 gram of sodium bicarbonate was given. This was in solution in physiological saline plus blood withdrawn from the peripheral system during the experiment. The syringe and needle used for injection of the bicarbonate solution had previously contained 1–1000 adrenin. This had been rinsed once with physiological salt solution but a substantial trace of the drug must have remained. The effect was striking. The heart rate increased from 185 to 220 per minute. At the same time the volume of

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to use a modification of Cullen's procedure for colorimetric determination (18). One centimeter of plasma obtained according to this method was pipetted under paraffin oil into small test tubes, 5 × 75 mm., containing 3 drops of brom thymol blue. The tubes were brought to 38°C. in a water bath. Following this procedure the tubes were placed in a comparator block where the colors were compared with those produced by standard M/15 phosphate buffers at 20 to 22° in the manner suggested by Clark (19). The 1 cc. samples of buffer solution also contained 3 drops of brom thymol blue. They were made up in steps of 0.1 of a pH unit so that differences of 0.05 of a pH unit could be detected. The readings so obtained were always more acid than expected. This was ascribable to the correction factor for protein, salt, and temperature errors. The magnitude of the combined errors was determined electrometrically and the correction estimated as 0.60 of a pH unit ±0.05.

In the gasometric determination the total CO_2 in solution was estimated by means of the Van Slyke constant volume blood gas apparatus using 1 cc. of plasma (20). "Alveolar air" samples, obtained by the method we have already described, were analyzed by the Haldane method (21). From these data the pH was estimated, using the following equations:

$$pH = P_K + \log \frac{(BHCO_3)_p}{(CO_2)_s} = 6.12 + \log \frac{(BHCO_3)_p}{(CO_2)_s}$$

where

$$(CO_2)_s = 760 - P_{H_2O} \times \frac{\%\ CO_2 \text{ alveolar air}}{760 \times 100} \times 0.935 \times CO_2 \times 100$$

$$= (760 - 50) \times \frac{\%\ CO_2 \text{ alveolar air}}{760} \times 0.935 \times 55$$

$$= 0.48 \times \%\ CO_2$$

$$(BHCO_3)_p = (\text{Total } CO_2) - (CO_2)_s$$

where $(BHCO_3)_p$ = Combined CO_2 in plasma
$(CO_2)_s$ = Dissolved CO_2 in plasma
or in cases where the pH was estimated from the CO_2 in whole blood

$$(BHCO_3)_p = 1.16\ (\text{Total } CO_2) \text{ blood}$$

The factors $P_K = 6.12$ and 0.935 were obtained from the paper of Van Slyke, Wu, McLean (22), while the factor 1.16 is estimated from the nomogram (23).

both ventricles measured by the cardiometer decreased 1 cc. Aortic output increased from 160 to 190 cc. per minute and the volume of blood in the lungs is decreased 6 cc. This decrease is apparently a constrictor effect due to adrenin plus an increase in rate of blood flow without an increase in volume inflow. Ventilation with a 20.43 per cent CO_2 gas mixture reduces ventricular output quite promptly but does not cause a release of the adrenin constriction in the pulmonary vessels. The general implication of the experiment is that adrenin in small dosage increases the rate of pulmonary blood flow and decreases the pulmonary bed. Owing to the possibility that the heart is more sensitive to adrenin than the pulmonary circuit it would seem possible to reduce the adrenin dosage so that a situation might appear in which blood flow was increased and the pulmonary vessels, being unaffected by the drug, were dilated by the increased blood flow. This type of adaptation would meet the necessities of exercise admirably. In our experiments with the heart-lung preparation, we have never been able to secure such a result. Adrenin has invariably emptied the lungs.

The H-ion concentrations of the blood in this experiment were determined gasometrically. The results were as follows:

	Blood gases	*pH gasometric*
1.	CO_2 = 40.8 vol. per cent O_2 = 16.83 vol. per cent	7.16
2.	CO_2 = 55.47 vol. per cent	7.25
3.	CO_2 = 60.79 vol. per cent O_2 = 15.02 vol. per cent	7.07
4.	CO_2 = 60.05 vol. per cent O_2 = 17.06 vol. per cent	7.26

The average difference between the colorimetric values and those calculated from the gasometric method gave the correction of 0.57 of a pH unit ± 0.05 to be added to the prime values.

Secondly, a sample of plasma was equilibrated with a mixture of 7.20 per cent CO_2 and 92.80 per cent hydrogen in a tonometer at 20 to 22°C. One portion was used for a colorimetric pH estimation as before. Another portion was placed in a Clark hydrogen electrode vessel and again equilibrated with the same gas mixture. The E.M.F. was then measured and the pH calculated from the following equation:

$$pH = \frac{E.M.F. + E_p - E_{cal}}{0.0001983\ T}$$

where

$$E_p = \frac{0.0001983\ T}{2} \log \frac{760}{pH_2}$$

The difference between the two values, 0.60 of a pH unit, coincided with that between the gasometric and colorimetric values within the limits of error. No claims to great accuracy can be made for the colorimetric method herein described. It is probably accurate to ± 0.05 of a pH unit. This was adequate for our purposes since we were interested in knowing in what pH range we were working and in making certain that the pH values of the plasma of our heart-lung preparations were comparable with those in anesthetized but otherwise normal cats. The method should not be used without modification where more precise measurements are required.

Here again there is no relation between pH and pulmonary blood volume.

Experiment III. October 30, 1925. Cat, weight 3.2 kgm. Figure 7.

Determination of the volume of blood in the heart and lungs with:

1. Change in ventilating gas from 4.97 per cent CO_2, 19.83 per cent O_2, and 75.21 per cent N to 4.91 per cent CO_2, 8.71 per cent O_2, and 86.38 per cent N.

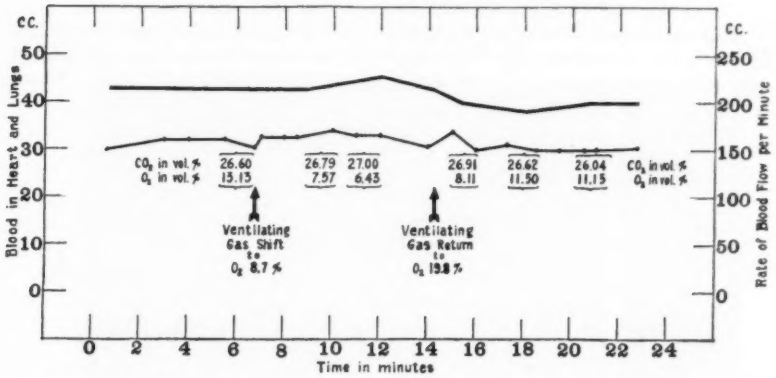

Fig. 7. Effect on pulmonary blood volume of decreasing blood oxygen. The system of charting is identical with figure 5.

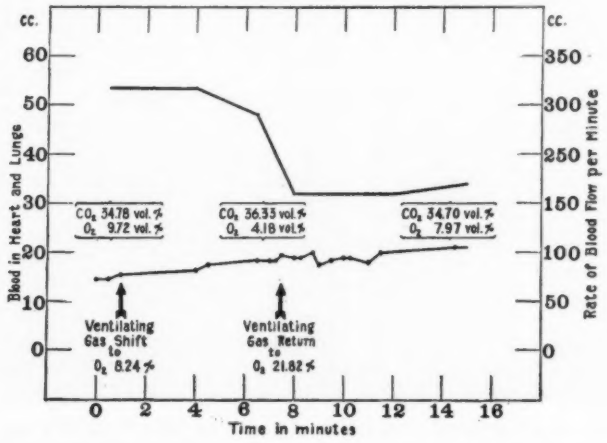

Fig. 8. Effect on pulmonary blood volume of decreasing blood oxygen to the point of causing cardiac failure. The system of charting is identical with figure 5.

In this experiment, shown in figure 7, the blood CO_2 is low throughout. Reduction of the blood oxygen, unaccompanied by serious alteration in blood flow, is without effect on pulmonary blood volume. The heart dilated somewhat during the low

oxygen period but the degree of oxygen want is not sufficient to cause a decided falling off in output. There is no suggestion of cardiac failure.

Experiment IV. November 6, 1925. Cat, weight 3.2 kgm. Figure 8.

Determination of the volume of the blood in the heart and lungs with:

1. Change in ventilating gas from 5.22 per cent CO_2, 21.82 per cent O_2, and 72.96 per cent N to 4.96 per cent CO_2, 8.24 per cent O_2, and 86.8 per cent N.

This experiment, shown in figure 8, differs from the preceding in that oxygen lack is severe, the oxygen falling to 4.18 volumes per cent in the blood. The heart dilated markedly, blood flow was reduced, and blood has accumulated in the lungs. There

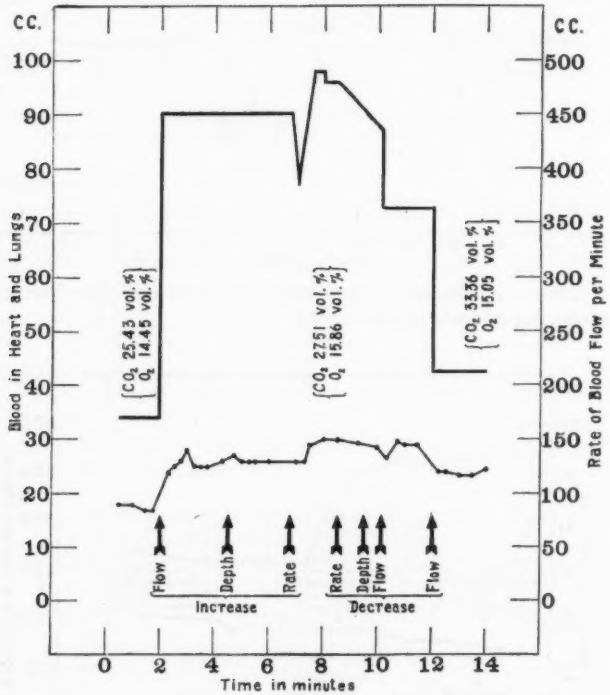

Fig. 9. Effect on pulmonary blood volume of increasing blood inflow into the right auricle followed by alterations in the artificial respiration. The method of charting is identical with figure 5.

was no recovery from this accumulation. The picture is the invariable one obtained when the left ventricle fails. The right ventricle has a very yielding vascular bed in the lungs and readily increases the volume of blood in the lungs without the necessity of withstanding much increase in pressure.

On the other hand the systemic arterial circulation is practically unchangeable under the circumstances of our experiment. One cannot contend that the right ventricle is composed of sturdier stuff than the left. It may often appear disproportionately able to endure strains but this appearance is rather an expression of the

"factor of safety" inherent in the size and extraordinary elasticity of the pulmonary bed.

Experiment V. November 24, 1925. Cat, weight 2.9 kgm. Figures 9 and 10.

Determination of the volume of blood in the heart and lungs with:

1. Changes in blood flow and in the rate and depth of artificial respiration. Ventilating gas held unchanged 10.0 per cent CO_2 and 90 per cent O_2.

After four readings of cardio-pulmonary volume, figure 9, the inflow clamp, *23*, in figure 1, was opened so as to increase aortic blood flow from 170 cc. per minute to

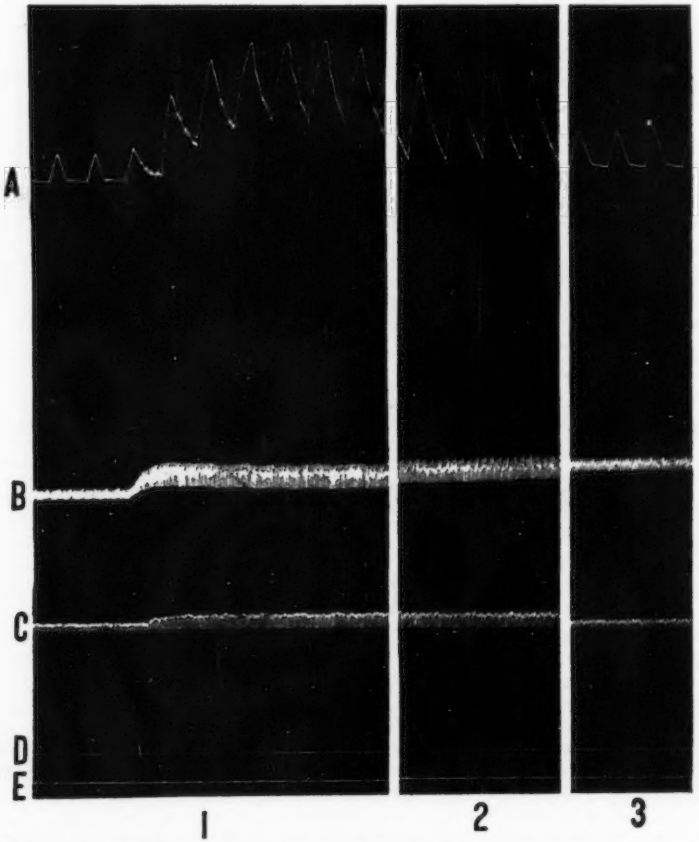

Fig. 10. Sections of kymographic record obtained in experiment 5. A, tracing of Brodie bellows recording blood flow; B, traced by a second Brodie bellows and recording volume of the ventricles through a capsule cardiometer; C, tracing of blood pressure by mercury manometer; D, base-line for blood pressure; E, time in 6-second intervals. The first slip of tracings records the increase in aortic blood flow from 170 to 450 cc. per minute, the second falls between the third and fourth arrows in figure 9 and the fourth after the last arrow in figure 9.

450 cc. per minute. This extreme increase in blood flow caused a rise in bi-ventricular volume of 1 cc., and in no way embarrassed the heart, a result reasonably apparent from the manner in which the blood flow is seen to remain constant in figure 9 and the heart to resist dilatation in figure 10. Reading from left to right in figure 9, the second arrow indicates the moment when the artificial respiration was increased in depth, rate being unchanged. This increase was from 8.75 cc. per respiration to

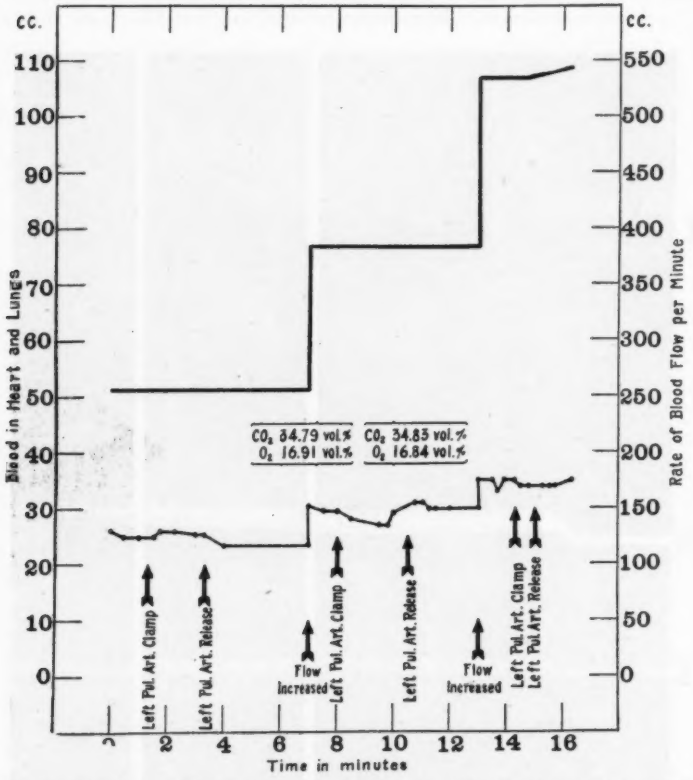

Fig. 11. Effect on aortic blood flow and pulmonary blood volume of clamping the left branch of the pulmonary artery (the blood supply to the left lung) under three different rates of blood flow. The method of charting is identical with figure 5.

27 cc.—an increase in minute volume from 262.5 cc. to 810 cc. There is a trifling increase in pulmonary blood volume. At the third arrow the rate of ventilation was increased from 30 to 46 respirations per minute, the volume of each respiration being left at 27 cc. This results, first of all, in a fall of aortic blood flow followed by a rise. The volume of blood in the lungs increases but the increase is synchronous with the augmentation of blood flow. It has not been possible to demonstrate an increase in pulmonary blood volume from increased artificial respiration without concomitant increased blood flow. We are at present unable to visualize just how the augmented

artificial respiration produces the increase in pulmonary blood flow which, in figure 9 shows itself in increased aortic flow, nor have we obtained significant increases in pulmonary blood volume when flow did not become larger.

It is the indication from our experiments that breathing movements of artificial respiration *per se* do not increase the amount of pulmonary blood to a noticeable degree but since normal hyperpnea is invariably accompanied by increased blood flow, an augmentation of pulmonary blood volume will invariably accompany it.

Experiment VI. January 13, 1926. Cat, weight 3.0 kgm. Figures 11 and 12.

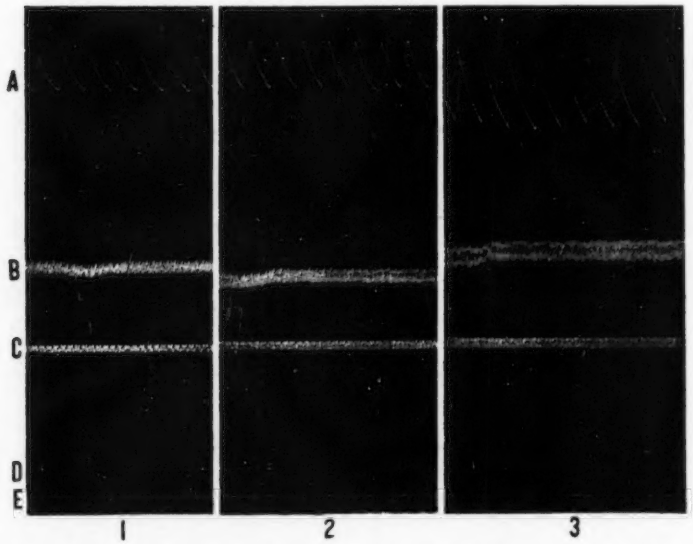

Fig. 12. Sections of kymographic record obtained in experiment 6. A, tracing of Brodie bellows recording blood flow in the aorta; B, traced by a second Brodie bellows and recording the volume of the ventricles through a capsule cardiometer; C, tracing of blood pressure by mercury manometer; D, base-line for blood pressure; E, time in 6-second intervals. The first slip of tracings records the first occlusion of the left branch of the pulmonary artery, the second slip the second occlusion and the third the final occlusion. Note the insignificant degree of cardiac dilatation which accompanies occlusion of the entire circulation in the left lung even with a very vigorous blood flow.

Determination of volume of blood in the heart and lungs with:

1. Complete occlusion of the left branch of the pulmonary artery under different rates of blood flow. Ventilating gas held unchanged.

The results of this experiment are given in figure 11. With the inflow clamp *23*, figure 1, adjusted so as to give an aortic outflow of 255 cc. per minute, clamping and release of the left branch of the pulmonary artery has no effect on aortic blood flow. In other words, at this rate of blood flow the right ventricle is entirely able to push the amount of blood which has been going through both lungs through the right lung alone, and in their turn the vessels of the right lung are able to receive the necessary increase in volume without offering serious resistance to the heart. When the blood

supply to the left lung is interrupted, a certain amount of blood is locked out of circuit in this lung and it becomes impossible to know just how large the effective pulmonary blood volume in the right lung may be.

At the third arrow, clamp *23*, figure 1, was released slightly and the aortic blood flow rises to 385 cc. The left branch of the pulmonary artery was again clamped and released, again with no diminution in aortic delivery.

On the sixth arrow a further increase in blood flow was induced and again the left branch of the pulmonary artery was clamped and released. Even with this extreme blood flow, the vessels of the right lung readily carry the entire load. Figure 12 is

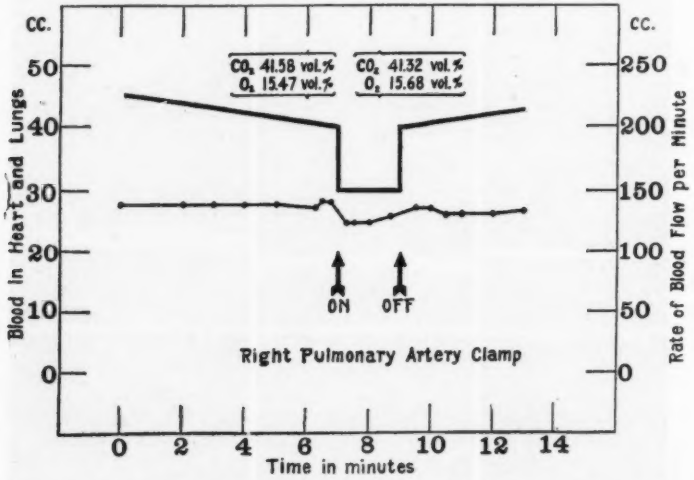

Fig. 13. Effect on aortic blood flow and pulmonary blood volume of clamping the right branch of the pulmonary artery. The method of charting is identical with figure 5.

the kymographic record obtained in this experiment. The upward marks on line D in 1, 2 and 3, indicate the moments when the left branch of the pulmonary artery was clamped.

At autopsy the left lung was not abnormal. The three occlusions were brief and ventilation was never interrupted. The experiment is a forcible illustration of the elasticity and the vascular area available in the normal lungs.

Experiment VII. January 18, 1926. Cat, weight 2.7 kgm. Figures 13 and 14.

Determination of the volume of blood in the heart and lungs with:

1. Complete occlusion of the right branch of the pulmonary artery. Ventilating gas held unchanged, CO_2 7.8 per cent, O_2 92 per cent.

Figure 13 shows the course of this experiment. When the right pulmonary artery is clamped all the blood reaching the left ventricle must pass through the left lung. The vascular bed in this lung is not large enough even though the rate of cardiac inflow is fairly low. A prompt reduction in aortic output takes place and there is a fall in the pulmonary blood volume. Figure 14 is the kymographic record. There is slight cardiac dilatation when the right branch is clamped but owing to the moderate inflow this does not become excessive.

Experiments VI and VII are of interest in that they show a decided difference in the size of the vascular bed in the two lungs of the cat. The right is larger and total obstruction on this side proportionately more serious.

Experiment VIII. January 8, 1926. Cat, weight 4.0 kgm. Figure 15.

Determination of the volume of blood in the heart and lungs with:

1. Compression of the pulmonary veins. Ventilating gas held unchanged, CO_2 7.5 per cent, O_2 92.5 per cent.

Compression of the pulmonary veins began on the first arrow, the clamp being slowly tightened until an obvious effect on blood flow was obtained. The drop in blood flow and the increase in pulmonary blood volume are simultaneous. Both are

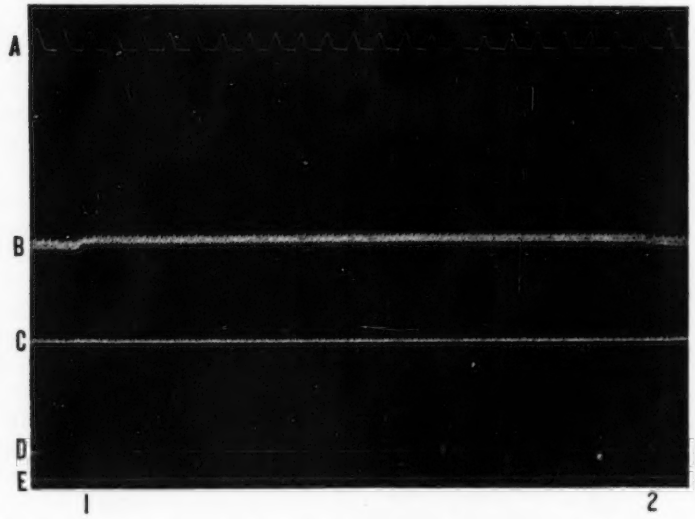

Fig. 14. Section of kymographic record obtained in experiment 7. A, tracing of Brodie bellows recording blood flow in the aorta; B, traced by a second Brodie bellows and recording the volume of the ventricles through a capsule cardiometer; C, tracing of blood pressure by mercury manometer; D, base-line for blood pressure; E, time in 6-second intervals. At the mark *1*, the right branch of the pulmonary artery was clamped, and at the mark *2* released.

arrested just before the second arrow when the clamp was again tightened. This further occlusion resulted in a progressive increase in pulmonary blood volume but the aortic output after an initial period of reduction rises markedly and does not fall until the third arrow which marks the final tightening of the pulmonary vein clamp. Between the second and third arrows the condition represents what is seen in a compensated case of mitral stenosis.

A final tightening of the clamp at the third arrow causes a further accumulation of blood in the lungs, but since the veins are completely occluded this cannot continue.

The conditions of the experiment are taxing to an extreme degree since in the heart-lung preparation inflow pressure on the right side of the heart continues unabated though outflow is wholly checked. This results in maximal development of pressure

in the pulmonary circuit and maximal increase in pulmonary blood volume. The maximal cardio-pulmonary blood volume at complete occlusion is 43.5 cc. The increase from 25.5 cc. to 43.5 cc. is in lung blood. In normal resting condition a large cat (4 kgm.), in this case, has 12 to 18 cc. of blood in his lungs. Taking 16 cc. as the normal, there is an addition of 18 cc. Owing to the rapidity with which this increase has been produced, a trifle over 4 minutes, the increase is intravascular and does not represent lost fluid in alveoli. Six per cent of the body weight is an average figure for the total blood volume in the cat (24) and on this basis the animal used in this

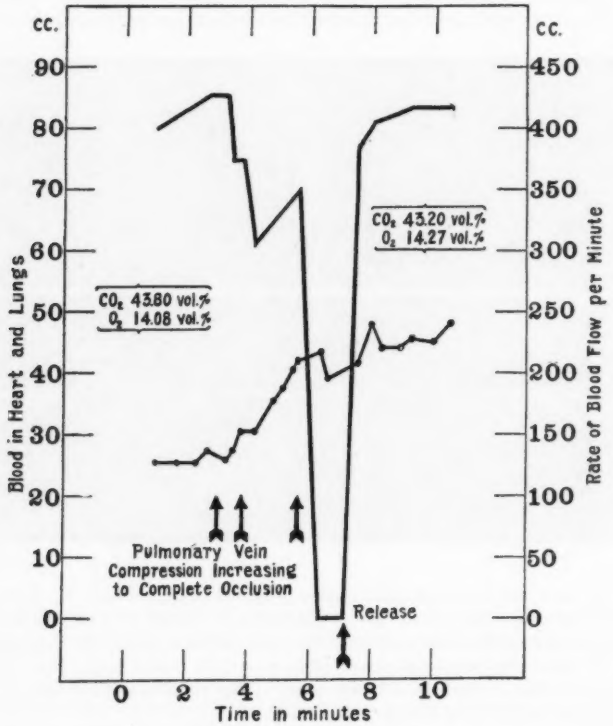

Fig. 15. Effect on aortic blood flow and pulmonary blood volume of clamping the pulmonary veins. The method of charting is identical with figure 5.

experiment had a total blood volume of 240 cc. The maximal pulmonary blood volume is 34 cc. or 14.2 per cent of the total blood volume, a lower figure than that obtained by Stewart (11) in his total obstruction experiments on dogs.

The percentage values, while of interest, are of less importance than the direct ones. Evidently the pulmonary vessels will carry slightly more than double the normal amount of blood for brief periods. This would be the natural expectation in view of the readiness with which the total blood flow may be accommodated by one lung.

The pulmonary vein clamp was released at the fourth arrow. There is an immediate rise in blood flow and a further slight increase in pulmonary blood volume which is clearly becoming progressive when the experiment was terminated. Since this state of affairs has bearing on the whole question of pulmonary blood flow, consideration of it is reserved for the final section of the paper.

Discussion. The experiments reported constitute a further expression of the passive position taken by the pulmonary circuit in the general phenomena of the vascular system. Although the character of the preparation used does not permit the possibility of reflex alterations in the calibre of the pulmonary vessels, the evidence for such regulation rests upon the unconfirmed observations of Sharpey-Schafer (8) on the depressor nerve reflex. This evidence unsupported by further control of blood flow into the heart is not of sufficient certainty to cause us to feel that the impressions we have gained are not in accord with the normal circumstances of pulmonary vascular adaptations.

The results of increasing carbon dioxide and decreasing oxygen are practically negative. There is no evidence that either of these measures causes more than an insignificant enlargement of the pulmonary blood volume. When oxygen lack, as in experiment IV, is carried to an extreme, blood does accumulate slowly in the lungs. In the heart-lung preparation with a capillary resistance of the type used in our experiments, the resistance offered to the left ventricle is unvarying and throughout the entire series of experiments has been kept as nearly as possible at 100 mm. Hg. When blood oxygen is abnormally reduced the left ventricle fails to meet this load before the right is seriously embarrassed. Blood may back up in the lungs to a very fair amount before pulmonary arterial pressure is measurably increased. This fact expresses the extreme elasticity of the pulmonary circuit and it accounts for the accumulation of blood in the lungs when aortic discharge is reduced in the early stages of left ventricular failure.

In experiment I where blood CO_2 and the hydrogen ion concentration of the blood are increased, the heart gives no indication of failure of output though the ventricular volume measured by the cardiometer increases slightly. Synchronous with this change the pulmonary blood volume increases 2 cc. It must, however, be pointed out that the figure for pulmonary blood volume contains auricular blood and that the auricles dilate somewhat under the influence of the CO_2. This auricular error, due to inability to completely enclose the whole heart in the cardiometer is unavoidable, and probably accounts for such augmentation of volume as the experiment records and thus leaves us with the safe conclusion that increases of CO_2 within limits which can appear in life are without significant effect on the calibre of the pulmonary vessels. The same considerations apply to moderate conditions of oxygen lack such as appear in experiment III.

In contrast to the carbon dioxide and oxygen experiments are those upon blood flow. Anrep and Bulatao (25), in a valuable paper, have explained the increase in pulmonary blood pressure which accompanies heightened aortic pressure, by the augmented pulmonary flow due to the enlarged volume of coronary blood. Our own experience, over many experiments in which pulmonary arterial pressure has been measured during increased cardiac inflow, confirms the emphasis these authors have placed on the effects of right ventricular output on pulmonary blood pressure.

Figures 5, 9 and 11 show the degree to which pulmonary blood volume depends on cardiac inflow and also show the volume changes which may readily occur. In figure 5 when the aortic blood flow advances from 267 to 534 cc. per minute, a figure which is lower than the pulmonary blood flow by the amount of the coronary flow, the pulmonary blood volume increases 6 cc. The heart dilates very little with this increase. On reducing the blood flow from 534 to 117 cc. per minute, the volume of blood in the lungs falls 13 cc. In any distensible system, such as the pulmonary circuit, increases in volume must be limited by the basic condition on which they are imposed. We have attempted in one case, experiment VIII, to indicate the actual amount by which the pulmonary blood volume may be augmented. In continuing experiments it is only possible to do this in terms of approximations. Pulmonary blood volume varies, dependent on blood flow at the moment and one can make no general statement covering the lung fraction of the total blood volume. The absolute figures, 12 to 18 cc. used as normal in experiment VIII are the outcome of a large experience and it is perhaps justifiable to employ them in that case which was designed to illustrate maximum pulmonary capacity. Our data permit us to do no more than picture the number of centimeters of blood which may be added to or taken away from unknown amounts of pulmonary blood during definite periods of time. They display the tendency of the changes which occur in the pulmonary circuit when blood flow increases and decreases but they do not give us the quantitative relation of increase and decrease to preceding volumes.

It is exceedingly difficult to visualize the state of affairs which must exist in the lungs throughout such changes as are shown in experiment I. If the blood flow through the lungs is markedly reduced the pulmonary blood volume falls. The question which at once arises is whether or not as many capillaries are transmitting blood in the second condition as in the first, and this question bears directly upon reactions in the intact animal since five-fold changes in cardiac minute volume are of frequent occurrence. In our opinion it is improbable that nervous regulation alters the size of the pulmonary bed to meet the conditions of the moment. No experiments exist in which the capillaries of the mammalian lung have

been observed under known and widely different conditions of pulmonary blood flow. Hall (4) reports direct observations on non-dependent portions of the rabbit and cat lung which have a qualitative bearing on the problem.

> In general, the flow in the larger arterioles is pulsatory, the cellular elements are densely packed while expansion and relaxation of the walls is not observed. In the smaller arterioles, the stream is no longer pulsating, the individual blood cells travelling in ranks of four to six. In the capillaries, the blood corpuscles travel in single file, and in a continuous stream, but the rate of flow varies in the vessels even in the network surrounding the same alveolus. In the smaller vessels, the stream is constant and a little more rapid than in the capillaries, individual cells being distinguishable. In the larger vessels, and the smaller veins, the stream shows a definite pulsation, the onward flow being reduced during systole and increased during diastole, suggesting a back transmission of pressure—changes from the left auricle.

These observations indicate that with rates of cardiac inflow which exist in animals with open chests and under a modified type of intratracheal insufflation, blood requires more time to pass certain vascular routes than others which offer less resistance. Depreciation of cardiac inflow is the great cause for a lowered head of pulmonary arterial pressure. It is justifiable to consider that Hall's report deals with a capillary circulation which, if anything, is below normal and under these circumstances it is evident that capillaries are in process of falling out of the conducting circuit and this tendency does not depend on active contractions but upon the simple fact that in the vast interconnecting complex of lung capillaries, certain paths, and these probably vary with position and with the character of respiration, are more difficult of transit than are others. It requires but a glance at reconstructions of the air sacs (26) to realize the manifold courses available for blood in the pulmonary capillaries and thus to appreciate the implication of Hall's observations on variation in capillary rates of flow.

One may assume that under rapid rates of blood flow all of the pulmonary capillaries are conducting blood and that the rate of movement in individual capillaries approaches equality, but that as blood flow into the right ventricle falls off, the easiest routes are chosen and with exceedingly low blood flows many capillaries contain blood which is practically not in motion or which is moved slowly into the pulmonary veins.

Recent observations on the blood flow through the lungs in man (27) indicate a tailing-out of columns of injected solutions as they pass through the lungs. These findings are not in entire accord with those of Stewart (11), (28), made in the early nineties, but as they appear through more delicate methods of observation they may be considered as representing the true state of affairs. One factor in the production of such tailing-out would seem to be this difference in flow rates in different capillary paths.

It is therefore our belief that when blood flow through the lungs is rapid and pulmonary blood volume increases, all available vascular routes are conducting blood and are somewhat distended. When flow falls and the pressure playing upon different capillary paths is low, then only the easiest routes transmit the effective blood current, blood moves sluggishly through the more difficult, and the total pulmonary blood volume decreases.

One may carry this conception of passive regulation of flow through different lung areas over into problems offered by pulmonary congestion. It is an ordinary observation in prolonged mammalian experiments in which the circulation fails slowly, that the dependent portions of the lungs are a dusky red and contain increased amounts of blood. In the same way dependent parts of the lung in man show passive congestion in cardiac decompensation. In both of these cases it seems evident that blood has been affected by gravity and moved in accordance with this force rather than with the onward impulse from the right ventricle. The veins of neighboring lung lobules anastomose freely. When the cardiac output becomes low, due to poor filling as in the case of the experimental animal, or when through left ventricular incapacity blood backs up in the lungs, there is a gravitational stuffing of dependent veins which eventually results in a practical stoppage of flow in dependent areas. Under such circumstances the actual pulmonary current passes through areas above the congestion, the rate of movement in the congested portion being variable and practically negligible in the most affected parts. In experiment VIII we have shown the most extreme degree of pulmonary congestion which can develop. This produces what on rough estimate we consider a doubling of pulmonary blood volume. It is noticeable that when the pulmonary vein clamp is removed, although the aortic output rises to the original level the pulmonary blood volume remains elevated. At autopsy the lungs were "heavy and bloody and showed patchy, dark, atelectatic areas." In the past three years we have had a good deal of experience with lungs which for experimental reasons have been placed in this condition. There is always a moderate amount of blood actually in the alveoli but in the main the retained blood is intravascular. When this retention is the result of such a radical procedure as in experiment VIII, rapid pulmonary vein compression with unrestricted inflow into the right ventricle, atelectatic areas appear with great promptitude. It is not difficult to understand the appearance of diffuse pulmonary areas heavily stuffed with corpuscles through which blood flow will be resumed but slowly. We are, however, unable to explain the readiness with which atelectasis tends to appear in regions acutely and heavily congested. There is probably a relation to the ability of air to enter the alveoli but

the manner in which this additional factor may operate leads us too far afield.

The fact that our experiments have shown little variation in pulmonary blood volume under different conditions of respiration unless accompanied by increase in blood flow apparently contradicts many observations which indicate the enlargement and lengthening of pulmonary capillaries during inspiration. We have referred to the literature upon this inspiratory increase in lung blood and our failure to obtain evidence of it may be taken to express differences between the normal negative pressure ventilation of the intact animal and the positive pressure ventilation used in these experiments. Possibly this is correct, but it is proper to point out that in actual measurements of lung blood in the inspiratory and expiratory positions no attention has been paid to possible augmentations and reductions in pulmonary blood flow which accompany the respiratory act. Divested of the increased blood flow which invariably accompanies hyperpnea in the normal intact animal we are of the opinion that the pulmonary blood volume would show but insignificant alteration.

The experiments on compression of the right and left branches of the pulmonary artery require but little comment. The greater effect on the right side was somewhat surprising to us and corrects an impression of equality between the two lungs which appears in an earlier paper (17) from this laboratory.

SUMMARY

1. A brief discussion of literature bearing upon pulmonary blood volume and its regulation is presented.

2. A method for measuring the cardio-pulmonary blood volume is described. This depends on determining the total blood volume in a heart-lung preparation in the cat and making a series of substractions of the volume in the systemic part of this reduced circulation. A correction obtained from cardiometer tracings permits subtraction of ventricular changes in volume from the original cardio-pulmonary total. The charted results express amounts of increase or decrease in pulmonary blood volume but do not give this total volume at any moment. These measurements contain an error since auricular blood is included in them.

3. The heart-lung preparation is novel in that the lungs are enclosed in the chest and that a new method for measuring blood flow is employed.

4. Alterations in blood carbon-dioxide and blood oxygen have no significant effect on pulmonary blood volume if cardiac-inflow remains constant and if no failure of the left ventricle occurs.

5. Alterations in the ventilation so as to hold the lungs in an advanced mid-position produce no increase in pulmonary blood unless accompanied by increase in blood flow.

6. Increase in inflow into the right ventricle is the only means falling within ordinary normal experiences which results in increase of the pulmonary blood volume.

7. When the left branch of the pulmonary artery is occluded the right lung gives free passage to the pulmonary blood even when cardiac inflow is greatly increased.

8. Occlusion of the right branch of the pulmonary artery indicates that the left lung is less in vascular size, since diminution in aortic output appears at once.

9. On clamping the pulmonary veins to complete occlusion without restriction of right ventricular inflow the lungs can be made to contain slightly more than double their original volume.

10. A discussion of what may be termed the passive regulation of pulmonary blood volume and blood flow is given.

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THE FAMILY RESEMBLANCE OF FEMALE RATS WITH RESPECT TO (1) THE AGES OF FIRST OESTRUS, AND (2) THE BODYWEIGHTS[1]

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In many laboratories where small numbers of animals are subjected to experimental study it is a common practice to reserve as "controls" a part of each litter under consideration. This practice is founded upon the assumption that littermates, because of a combination of genetic, environmental, and other factors resemble each other more closely that non-littermates from the same colony grown under similar conditions. And, because of this closer resemblance, they furnish a better basis from which to estimate the influence of specific factors of diet, husbandry, test procedure, etc., than that afforded by animals taken at random from the colony. How superior this basis is, however, is usually a matter of conjecture since very little is known concerning the actual family resemblance of rats. Hence it is desirable to ascertain the amount of resemblance normally found in any trait unders consideration since this information may be of some importance in differentiating chance variations between control and experimental groups from small differences experimentally induced.

Through the kindness of Dr. H. M. Evans, University of California, records on oestrual phenomena and growth were made accessible for a study of the correlation of the ages of first oestrus and the body weights of female rats of various ages. From a relatively simple statistical study of these data one is able to estimate roughly the advantages secured, if any, by selecting littermates rather than non-littermates as controls in studies of oestrual age and body weight at different ages.

Source of data. The rats from which the data of this study were collected are descendants of one wild brown male and a small group of albino

[1] Certain expenses incurred in the collection and statistical manipulation of these data were met from funds granted by the National Research Council through the Committee for Research on Sex Problems. Indebtedness to Dr. H. M. Evans for the privilege of using data from his laboratory, to Prof. J. E. Coover for suggestions on the statistical treatment of the data and to Mrs. Mary L. Hulbe for clerical aid is gratefully acknowledged.

females.[2] As to coloration, some were black, some white, some gray, and others hooded. Although they are many generations from the original stock at the present time, no attention has been given to the selection of particular strains with respect to coat color. From the standpoint of physical growth and vigor, however, a small amount of selection in the direction of heavier and healthier animals has probably occurred.

A comparative study of the ages of first oestrus in these mixed strains and in pure albino strains secured from the Wistar and Stanford (Slonaker) colonies has not revealed any significant differences between the groups, hence conclusions drawn from the data of this study would seem applicable to pure albinos as well as to the mixed strains herein represented. Whether the same can be said for body weight is a question we cannot answer at the present time.

The litters were chosen in a random order from a large colony maintained on the same diet, except that those containing only one female were rejected because they did not afford a basis for the comparison of sisters. In all, 95 litters[3] totaling 337 animals were studied. The number of females in each litter varied from 2 to 6. In order to equalize the nutrition of young as much as possible during the nursing period the total size of the larger litters was limited to six individuals by sacrificing new-born males.

Diet. The diet on which the mothers and young were maintained consisted of the following mixture of raw materials:

Standard diet I (Evans and Bishop, 1922)

Whole wheat	67.5
Casein	15.0
Whole milk powder	10.0
Calcium carbonate	1.5
Sodium chloride	1.0
Butter fat	5.0
	100.0

Ascertaining the age of first oestrus. By daily inspections of vaginal smears (Long and Evans, 1922) the age of each female at the onset of oestrus was ascertained. These examinations were begun when the young females had reached the age of 30 days and were continued daily thereafter or until the first oestrus had appeared. Doctor Evans and his coworkers have satisfactorily demonstrated that reliable data on the onset

[2] For details as to the environmental conditions under which these animals were reared and studied, methods of feeding, daily routine of examinations, etc., the published reports of Long and Evans (1922) and Evans and Bishop (1922) should be consulted.

[3] Very few, if any, of the litters are offspring of the same parents.

of oestrus can be obtained by the method of smear examination. For our purpose, therefore, suffice it to say that two competent observers inspecting the same vaginal smear seldom disagree as to the stage of oestrus typified by a given specimen and when disagreement does occur probably a variation of only one day in the date of oestrus recorded would result. This small amount of disagreement, occurring very rarely, may be considered negligible for this study.

Table 1 gives the distributions of ages of first oestrus for litters of from 2 to 6 females together with the arithmetical means and standard deviations of these distributions. In figure 1 these distributions are graphically represented. Whether the number of females per litter is 2, 3, 4, 5 or 6, the means vary only slightly and by insignificant amounts. Likewise the standard deviations of these distributions, with the exception of that

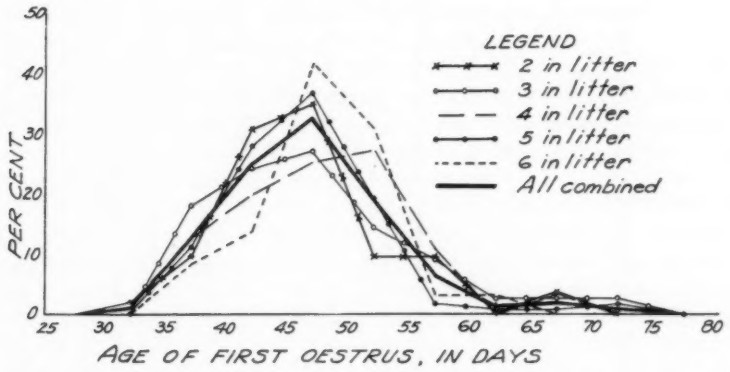

Fig. 1. Frequency polygon showing the distribution of ages of first oestrus for rats taken from litters containing 2, 3, 4, 5 and 6 females.

for the distribution of animals coming from litters of six individuals, which is slightly smaller than any of the others, are quite similar in magnitude. Although the difference between the standard deviation of the distribution for "six in the litter" and both "three in the litter" and "four in the litter" is barely significant mathematically, it is not sufficiently great to warrant special emphasis because of the small number of litters represented in the distribution designated "six in the litter." This distribution comes from only 6 litters whereas 26 and 14 litters respectively are considered in the distributions for "three" and for "four in the litter." With the relatively small number of litters there is less chance of approximating a normal distribution of ages of first oestrus than in the distributions embodying the greater numbers. Henceforth the number of females per litter has been disregarded in all computations pertaining to the age of oestrus

TABLE 1

Distributions of the ages of first oestrus for the females of 95 litters

AGE OF FIRST OESTRUS	2 FEMALES IN LITTER	3 FEMALES IN LITTER	4 FEMALES IN LITTER	5 FEMALES IN LITTER	6 FEMALES IN LITTER	TOTAL
days						
73				1		1
72				1		1
71		1				1
70		1				1
69						
68			1			1
67	1	1				2
66		1		1		2
65	1		1			2
64		1				1
63				1	1	2
62						
61						
60		1				1
59	1	2	1			4
58	1	2		1		4
57	1		1			2
56	2		1			3
55		3	3	1	1	8
54		1	4	4		9
53	2	2	3	1	1	9
52		2	1	7	5	15
51		5	3	6	1	15
50	3	1	4	4	4	16
49	1	6	3	4		14
48	5	3	3	8	3	22
47	4	5	3	9	5	26
46	2	3	4	11	2	22
45	6	4	1	10	5	26
44	2	7	3	7	1	20
43	3	5	2	8	3	21
42	4	3	3	7		17
41	4	3	1	6		14
40	3	1	2	4	1	11
39	1	3	1	4	1	10
38	3	2	2	2	1	10
37		3	1	6	1	11
36	1	2	1	1		5
35		4	2			6
34						
33	1		1			2
Total number of females	52	78	56	115	36	337
Mean age of oestrus	46.2	47.2	47.6	46.4	47.4	
P.E. mean age oestrus	0.644	0.349	0.648	0.398	0.564	
S.D. distribution	6.8	8.16	7.2	6.3	5.01	

because it does not appear to have sufficient significance to the age of puberty to justify special consideration.

Individuals with ages of first oestrus varying greatly from the others of their respective litters. The age of first oestrus in a relatively small number of cases diverges widely from the mean of their respective litters. Eleven animals are sufficiently outstanding in this respect to deserve special consideration. On the average their ages of first oestrus are approximately twenty days beyond the mean for their littermates. With but 3 exceptions these individuals make up the entire scattering of animals with oestrual ages falling later than 60 days. (See distributions of table 1.) Naturally one raises the question as to whether these variations arise from adventitious circumstances of environment,—ill health, dietary deficiency or special handicap accidentally imposed upon them but not upon their littermates. If so, they are incomparable with the latter and should be discarded from the group. Available information on this point enables us to consider only a few points pertinent to the question.

In so far as possible all animals were given equal care throughout the period of study and since experienced technicians handled each animal daily there is little likelihood of an acute or chronic illness having escaped detection. Animals with detectable signs of illness were dropped as a matter of routine in the conduct of the experiment.

With respect to the possibility of inanition, only negative evidence has been found. The animals were allowed to feed ad libitum on a well balanced ration (Evans and Bishop, 1922) and the ingredients of this mixture were so finely ground and thoroughly mixed that they could not select or reject particular particles thereby depriving themselves of necessary elements of the ration. The average body weight of these individuals between the ages of 30 and 80 days is only slightly lower than that of their littermates. The data on this point are given in table 2. Comparisons within litters show that the body weights of some of these extreme cases fall below those of their littermates consistently throughout the prepubertal period and that the weights of others equal or exceed those of their littermates. These data do not strongly suggest general inanition as a probable cause of retarded puberty in the eleven individuals, although, to be sure, they do not positively rule it out. Evans and Bishop (1922) have demonstrated that it is possible to have a qualitative form of inanition which delays the first oestrus without manifesting itself by retarded bodily development for a relatively long period of time.

Before passing from this subject, one additional abnormality of these extreme cases should be noted. For them there is an interval of 12.1 days, on the average, between the rupture of the vaginal plate and the onset of the first oestrus. For the remaining 325 animals the average interval is only 1.49 days, and an interval of 12 or more days occurs in

only four instances. This relatively long separation of first oestrus and rupture of the vaginal plate strengthens our suspicions that these individuals are not comparable with their littermates with respect to age of first oestrus, but, until better understood, does not give unequivocal grounds for dropping them from further consideration. For this reason

TABLE 2

Body weights of females in which the oestrual ages vary greatly from the mean oestrual ages of their litters together with the average body weights of their littermates

	NUMBER IN LITTER	AGE IN DAYS AT FIRST OESTRUS	INTERVAL BETWEEN RUPTURE OF VAGINAL PLATE AND FIRST OESTRUS	BODY WEIGHTS IN GRAMS BETWEEN AGES OF 30 AND 80 DAYS					
				30	40	50	60	70	80
Disparate one.....	1	65	0	54	83	108	121	130	175
Mean of litter.....	1	46	0	63	93	116	137	155	156
Disparate one.....	1	67	12	71	104	107	150	164	181
Mean of litter.....	1	43	3	66	99	135	150	172	182
Disparate one.....	1	71	18	69	83	112	129	160	176
Mean of litter.....	2	46	1	79	119	145.5	171	198.5	226
Disparate one.....	1	70	15		105	132	165	185	196
Mean of litter.....	2	50.5	0		105	131	159	181.5	190
Disparate ones.....	2	66.7	7.5			106.5	125	134	142
Mean of litter.....	1	44	0			119	134	142	135
Disparate one.	1	65	14	65	103	142	166	185	190
Mean of litter.....	3	43.3	0	62.66	105.3	126	160	183.3	192
Disparate one.....	1	68	14		100	128	138	160	172
Mean of litter.....	3	46.6	1		102.66	140	166.3	187.3	197.6
Disparate one.....	1	72	23		101	132	160	180	200
Mean of litter.....	4	46.75	0.5		100	126.75	157.75	183.5	198.5
Disparate one.....	1	73	19	53	78	100	117	125	139
Mean of litter.....	4	47.5	0.5	58.5	94.75	120.5	141.25	148.75	182
Disparate one.....	1	63	3	69	95	120	145	166	174
Mean of litter.....	4	42	0	76	107.5	138.75	159.25	180.5	191
Mean of all littermates of disparate ones.....		45.63	0.48	67.53	103.04	130.60	155.72	175.67	190.39
Mean of all disparate ones.....		67.95	12.1	63.66	94.67	117.64	140.09	156.64	171.54

it has seemed desirable to calculate correlations both with them present and with them absent from our scatter diagrams.

Methods of comparing littermates. Two relatively simple methods of pairing littermates have been employed for the purpose of computing coefficients of correlation of the ages of first oestrus and of the body weights of sisters. The operation of these methods is as follows:

1. Method A: Each animal considered only once. Assume that we are dealing with a litter of four females designated by the numbers 1, 2, 3, 4. A simple method of comparing them is provided by pairing 1 with 2 and 3 with 4. Since only chance factors have operated in the numbering of individuals of the litter this grouping is probably as good but, in general, no better than any other grouping such as 1 with 3 and 2 with 4, etc., providing each animal is considered but once in the comparison. If the litter consists of any odd number of females one case should be dropped since to use it in the pairing would necessitate the repeated use of one of the others.

2. Method B: All possible pairs. Again consider a litter of four individuals numbered 1, 2, 3 and 4. To secure the maximum number of pairs without duplicating any combination, let 1 be paired with 2, 1 with 3, and 1 with 4; let 2 be paired with 3, and 2 with 4; and let 3 be paired with 4. By this method of grouping six different comparisons of littermates may be made from a litter of four individuals without duplication of pairs. Similarly from a litter of two, one pair is obtained for comparison; from a litter of three, 3 pairs; from a litter of five, 10 pairs; and from a litter of six, 15 pairs. Although for obvious reasons method B would be impracticable for actual laboratory procedure, there is no apparent objection to its use in the present study, since the distributions of oestrual ages of litters of different numbers of females has been found to be so very similar with respect to average and variability. The method is to be recommended because it provides a means of making all possible pairings between sisters and thereby increases the number of comparisons over that secured by method A. This increase in the number of pairs will increase the reliability of the coefficients of correlation for the trait under consideration. Both methods A and B have been used and the data for each will be presented.

Correlation of oestrual ages of siblings: The correlation of first oestrus for the littermates of this study have been calculated both with the 11 divergent cases present and with them omitted from the distributions. As will be seen there is reason for excluding them from the scatter diagrams because their positions in both frequency distributions show them to be heterogenous, and their influence upon the coefficient of correlation is spurious.

Table 3 is a scatter diagram of the oestrual ages of littermates paired

according to method A (animals used only once) and with the 11 divergent cases omitted. An inspection of the data of this table shows that the correlation of oestrual ages is very low. That is to say, there is only a slight tendency for all members of a given litter to have relatively early ages of oestrus, approximately average ages of oestrus, or relatively late ages of oestrus. As calculated, the coefficient of correlation (products-moment) is 0.235 ± 0.055. This coefficient is over four times the amount of its P. E., and through the operation of chance factors might be expected to occur once in about 143 instances of similar correlations. Hence we may safely conclude that there is a small amount of correlation between the ages of littermates at the time of first oestrus. On theoretical grounds it is reasonable to assume that a coefficient of approximately zero will be gotten if each female of table 3 is paired with an individual of a different litter. Tests of the foregoing assumption made with the data of this study prove that it is valid. (r by method A is -0.12 ± 0.051; r by method B is: $-0.11 = 0.044$. Neither coefficient is mathematically significant.)

Table 4 is a scatter diagram for all possible pairs of littermates grouped according to method B and with the divergent cases dropped out. The number of pairs is raised from 135, secured by method A, to 397 pairs, secured by method B. The coefficient of correlation for table 4 is 0.219 ± 0.032. Although this coefficient is not significantly different from the former it is much more reliable. The probability of its being a chance correlation is about 1 in 300,000 instances. Since the coefficient obtained from table 4 is based on all the possible pairings of littermates it would seem to represent the correlation for the animals of this group more adequately than that obtained from table 3.

With the 11 extreme cases of late oestrus included in the scatter diagrams of tables 3 and 4, the coefficients of correlation for the tables are 0.195 ± 0.054 and 0.121 ± 0.033, respectively. Their presence in the scatter diagrams reduces the sizes of the coefficients of correlation because they make the regressions slightly curvilinear, in which case the coefficients of correlation obtained by using the products-moment formula are somewhat too low[4] (Kelley, p. 238). Each of these coefficients is mathe-

[4] The correlation ratios (Kelley, p. 238) for the scatter diagrams with all extreme cases included have been calculated for the purpose of determining whether the non-rectilinearity of the regressions is sufficiently marked to make the use of the products-moments formula for the calculation of correlations unwarranted. For the data obtained by pairing method A, the correlation ratio of x on y is 0.253 ± 0.053; and for y on x it is 0.239 ± 0.055. For the same data the coefficient of correlation obtained by the products-moment formula is 0.195 ± 0.054. Although the correlation ratios are slightly larger the difference is not mathematically significant. For the data obtained by pairing method B the correlation ratio of x on y is 0.143 ± 0.025; and for y on x is 0.171 ± 0.025. The coefficient obtained by the products moment formula is 0.121 ± 0.033. The difference is not mathematically significant. In the light of these calculations it would seem that the use of the products-moment formula for the calculation of the coefficients of correlation is justified.

TABLE 3

Correlation of oestrual ages of littermates. Littermates paired only once (method A)

OESTRUAL AGE OF FIRST FEMALE OF PAIR	OESTRUAL AGE OF SECOND FEMALE OF PAIR																TOTAL
	33–34 days	35–36 days	37–38 days	39–40 days	41–42 days	43–44 days	45–46 days	47–48 days	49–50 days	51–52 days	53–54 days	55–56 days	57–58 days	59–60 days	61–62 days	63–64 days	
days																	
61–62																	
59–60			1			1											2
57–58							1										1
55–56						1					1						2
53–54				2		1	2	2	1	1		1					10
51–52		1				3	4	2	1	1			1			1	14
49–50				1	1	1	3	2	2	4	1	1	2				18
47–48			2	2	3	2	4	7	1			2					23
45–46				1	4	5	1	3	3	2		1	1	2			23
43–44					1	1	3	2		3							10
41–42		1	1		3	2	1	1			1						10
39–40			1	2	1		1	1		1							7
37–38		1	4		1	1											7
35–36		1	1	1			1	1		1							6
33–34						1						1					2
Total.....		4	10	9	14	19	21	21	8	13	3	6	4	2		1	135

r (products-moment) = 0.235 ± 0.055

TABLE 4

Correlation of oestrual ages of littermates (method B). All possible pairs of littermates

OESTRUAL AGE OF FIRST FEMALE OF PAIR	OESTRUAL AGE OF SECOND FEMALE OF PAIR															TOTAL
	33 days	35 days	37 days	39 days	41 days	43 days	45 days	47 days	49 days	51 days	53 days	57 days	59 days	61 days	63 days	65 days	
days																	
63							1		1	1							3
61																	
59								1									1
57						2			2	1	1						6
55						1	1	1	2	1	3	1				1	11
53				2		3	5	4	4	4	1	2				2	27
51		1		3	5	8	7	10	10	4	4	1	1		1	1	56
49		1	2	2	2	3	4	5	3	7	1	2	1				33
47		1	3	4	5	5	5	20	5	5	6	4					63
45		1	1	5	6	16	12	10	8	5	1	2	1	2			70
43				6	4	8	7	7	4	7	1						44
41		1		6	3	5	2	6	1	4	2						30
39				1	1	2	2	3	1	2	1	1					14
37		1	2	3	2	4		5	3	3	1						24
35		3	2	1			2			1	2						11
33						1	1				1	1					4
Total.....		9	10	33	28	58	49	72	44	45	25	14	3	2	1	4	397

r (products moment) = 0.219 ± 0.032

matically significant, however, although too low to truly indicate the resemblance for the major part of the group because of the spurious influence of the 11 heterogeneous cases.

Practical advantages gained by the use of littermates as controls in studies involving ages of first oestrus. These coefficients of correlation throw some light on our inquiry as to the practical advantages derived from the use of littermates as controls in experiments involving the ages of first oestrus. If an approximately perfect correlation, i.e., 0.9999 plus, were found between the oestrual ages of littermates reared under the same laboratory conditions, we should then be able to say that even small differences be-

TABLE 5

Correlation of body weights of sisters at the age of 30 days (method A used in pairing littermates)

WEIGHT OF FIRST FEMALE OF THE PAIR	WEIGHT OF SECOND FEMALE OF THE PAIR													TOTAL
	30 grams	35 grams	40 grams	45 grams	50 grams	55 grams	60 grams	65 grams	70 grams	75 grams	80 grams	85 grams	90 grams	
grams														
90												1		1
85										2				2
80									2		1			3
75							1	2	1	6	2	1		13
70					1		5	4	5	2				17
65						1	4	10	6	3			1	25
60					2	5	7	7	2					23
55		1		1	2	6	6	2	1					19
50					5	1	4							10
45		1		2	2									5
40					2		1							3
35														
30														
Total.....		2		3	14	13	28	25	17	13	3	2	1	121

r (products moment) = 0.724 ± 0.029

tween the oestrual ages of individuals of the control and experimental groups were due to the special conditions imposed upon the latter but not upon the former. But with a coefficient of correlation no higher than 0.2, or thereabout, the family resemblance with respect to oestrual age is so slight that differences between the mean age and the variability of the two groups of littermates are almost as great as between two groups of non-littermates selected from the same group. In this case one can attribute differences between individuals of the control and the experimental groups or differences between the groups as a whole to the special conditions to which the experimental group was subjected in the course of the

experiment rather than to chance variations only when these differences are large and fall beyond the normal range of variability for the controls. From this it may be inferred that, in studies involving the age of first oestrus, one gains only a very slight advantage as a result of equating the

TABLE 6

Correlation of body weights of sisters at the age of 30 days (method B used in pairing littermates)

WEIGHT OF FIRST FEMALE OF THE PAIR	WEIGHT OF SECOND FEMALE OF THE PAIR													TOTAL
	35 grams	40 grams	45 grams	50 grams	55 grams	60 grams	65 grams	70 grams	75 grams	80 grams	85 grams	90 grams	95 grams	
grams														
95														
90											1			1
85								1	3					4
80						2	2	2		2				8
75						3	5	13	18	5	1			45
70				3	2	11	17	12	7	2				54
65					3	18	26	22	9	1				79
60				3	14	37	28	10	2	1				95
55		1	5	10	16	29	5	4						70
50		1	1	14	5	11								32
45	1	2	3	7		1								14
40		1	2	3		1		1						8
35														
Total.....	1	5	11	40	40	113	83	65	39	11	2			410

r (products moment) = 0.687 ± 0.017

TABLE 7

Correlation of body weights of littermate rats at the ages of 30, 40, 50, 60, 70 and 80 days

AGE	r, METHOD A		r, METHOD B	
	No. pairs	r	No. pairs	r
days				
30	122	0.724 ± 0.029	410	0.683 ± 0.0169
40	138	0.565 ± 0.039	483	0.533 ± 0.022
50	141	0.480 ± 0.044	493	0.469 ± 0.0237
60	143	0.454 ± 0.045	484	0.407 ± 0.0256
70	130	0.419 ± 0.049	430	0.443 ± 0.0261
80	129	0.420 ± 0.049	407	0.439 ± 0.0266

factor of family resemblance in oestrual age by reserving littermates as opposed to non-littermates for the control over the experimental group.

Correlation of body weights of sisters at the ages of 30, 40, 50, 60, 70 and 80 days contrasted with the correlation of oestrual ages. Body weights of

the females considered in this study were recorded at five day intervals after the age of 20 days. By correlating their weights between the ages of 30 and 80 days we have secured additional measures of family resemblance with which to contrast the resemblance with respect to oestrual age. These coefficients of correlation will be of further interest because they roughly indicate what advantage is gained by reserving littermates as controls in experiments wherein the interpretation of experimental data involves the comparison of body weights of control and experimental animals. The weight relationships at the age of 30 days are illustrated by the scatter diagrams of tables 5 and 6, the data for which were obtained by pairing animals by methods A and B, respectively. For the data of table 5 the coefficient of correlation is 0.724 ± 0.029; that for the data of table 6 is 0.687 ± 0.017. Although the difference between the magnitudes of these coefficients is not significant, the latter, considered in relation to its P.E., is more reliable.

The coefficients of correlation for weights of littermates at the ages of 30, 40, 50, 60, 70 and 80 days are given in table 7. Since the scatter diagrams for the ages beyond 30 days do not present points of special interest not already illustrated by table 5 and 6 they have been omitted.

As may be observed from a consideration of the data of table 7, the coefficients for a given age obtained by pairing method A do not differ greatly from those derived from method B, but the reliabilities of the coefficients from the latter are much higher because of the greater number of pairs involved. The greatest amount of resemblance in body weight for these siblings occurs at the age of 30 days. Beyond that age the coefficients drop with each ten day interval until the age of 50 days is reached after which they differ by insignificant amounts. Whether the amounts of correlation for ages below 30 days is still higher than at 30 days or whether beyond 80 days they tend to approximate those of 60, 70 and 80 days, are questions we cannot answer because of the limited data at hand. It is safe to conclude, however, that throughout the early ages of life the family resemblance for female rats is sufficiently high to indicate a considerable advantage gained by selecting littermates as controls in experiments involving the comparison of body weights.

The discrepancy between the correlations for oestrual age and body weight before and after puberty is relatively great. At the age of 30 days the correlation for the latter is more than three times that for oestrual age; at 50 days of age, approximately average age of puberty, it is more than twice as great; and at 80 days, which is beyond the extreme age of puberty for all animals of the group, it is only slightly less than twice as large. At each of the ages studied, these differences are mathematically significant and sufficiently large to suggest strongly that certain fundamental differences in the hereditary and environmental factors underlying family

resemblance are present and should be given special consideration in future investigations. Although the present study does not furnish clews as to the nature of these hereditary and environmental factors or indicate their relative potency in producing the differences reported, it does set forth the relationships as they now are. For this reason it should serve as a point of departure for special experiments designed to reveal the nature and potency of the factors contributing to the difference in family resemblance between oestrual age and body weight.

SUMMARY

This study is concerned with family resemblance in female rats with respect to 1, the ages of first oestrus, and 2, the body weights at various ages. Observations were made on 337 individuals, comprising the female offspring of 95 litters. These data show that for ages of first oestrus the variability is almost as great for littermates as for non-littermates. Body weights, on the other hand, are much more variable among the latter than among the former. For ages of first oestrus in littermates the most representative coefficient of correlation found was 0.22 ± 0.032. The coefficients for body weights range between 0.68 ± 0.017 at the age of 30 days to 0.44 ± 0.027 at the age of 80 days.

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SLOW AND RAPID VARIATIONS IN THE MINIMAL STIMULUS REQUIRED TO EXCITE CORTICAL MOTOR AREAS

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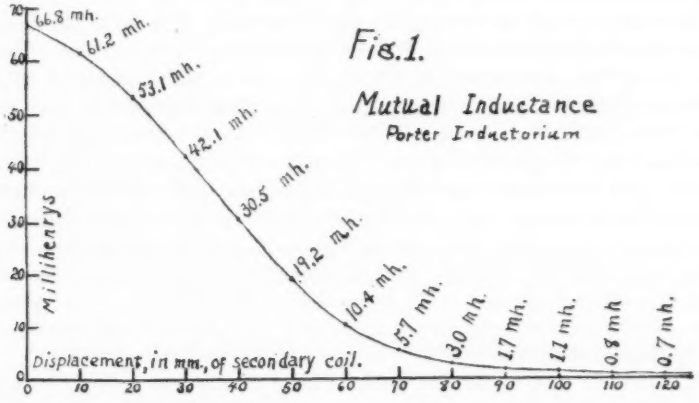
The present investigation is an outgrowth of some attempts to determine the effects of strychnin solutions applied locally to cortical motor areas for the legs of dogs. Preparatory to the application of strychnin, many tests were made to determine the minimal strength of induced current required to excite an area sufficiently to cause a reaction of the leg. The variations in the minimal stimulus for a normal area, i.e., before strychnin was applied, were remarkably small in most cases. In some, however, the variations were so large and complex that it was considered a waste of time to attempt to ascertain strychnin effects. Such extraordinary irregularities developed especially early in certain dogs which were diseased and emaciated. In some instances, although no rapid or abrupt variations occurred, there was a slow increase or decrease in the minimal stimulus value for an hour or more before strychnin was applied. There seem to be, therefore, two distinct types of variations, the slow and the rapid, which are independent of strychnin action.

The present investigation concerns the nature and causes of the slow and rapid variations in the minimal strengths of current required to excite cortical motor areas of dogs and cats. The experimental animals were nineteen dogs and three cats. Strychnin was not used. The results from healthy, well-nourished, adult dogs only, will be reported in the form of curves.

METHODS. Trephine openings were made over the cortical motor areas for the hind or front legs of a dog. Rigid frames were fastened to the skull to hold platinum electrodes in position on each of the cortical areas. The stimulating current was from the secondary coil of the Porter type of inductorium in all cases. The inductorium used in stimulating the left cortical area was mounted in an upright position, and the secondary was suspended above the primary coil by means of a strong thread that passed a few times around the shoulder of a work adder and then to and several times around an electrically driven rod. This rod could be set revolving or stopped at any time by throwing a lever. As it revolved, the secondary approached

the primary coil at the rate of approximately two millimeters per second (exactly 2.017 mm. per second) until a slight flexion of the leg occurred, then the falling of the coil stopped suddenly. This movement of the leg increased the tension on a thread which tripped a delicately set lever that fell and suddenly stopped the motion of the work adder and the secondary coil. The primary current was then broken. The distance between the coils was obtained by reading an especially arranged millimeter scale on the apparatus. The value was later translated into millihenrys by referring to a curve of mutual inductance plotted for the Porter inductorium (fig. 1).

The apparatus for the left cortical area was duplicated for the corresponding area in the other hemisphere. The same electrically driven rod worked both of the secondary coils. These coils ceased moving at the same or at separate times, depending on whether flexion of the legs



occurred simultaneously or separately. In preparing for a double observation, the electrically driven rod was thrown out of gear and turned by hand in the reverse direction until the secondary coils were raised as high as they would go, then the levers at the work adders were carefully adjusted and the secondary coils were set in motion at the same time that the primary currents were closed.

In some of the experiments, only one trephine opening was made and only one inductorium was used to stimulate the cortex.

The mutual inductances that were determined for the various centimeter positions of the secondary coil of the Porter inductorium, when the amperage and rate of interruption of the primary current were constant, are especially indicated in figure 1. The intermediate values were estimated by drawing a smooth curve through the thirteen points that were experi-

mentally determined. The features of this curve indicate clearly the advisability of translating the number of millimeters of relative superposition of the coils into millihenrys. The feature of chief interest is the rather sharp bend between sixty and eighty millimeters.

Since, by definition, the millihenry is the practical unit of mutual inductance of a pair of circuits when the rate of change of one milliampere per second in one causes an electromotive force of one millivolt in the other, the entire curve of mutual inductance is necessarily lowered or raised a certain amount if the rate of interruption of the primary current is altered. It was imperative, therefore, that special care should be taken to keep the rate of interruption and for obvious reasons the amperage of the primary current constant while investigating the reactions of any one animal. Generally, the amperage was tested and regulated at thirty minute intervals.

The motion of the secondary coil (2.017 mm. per second in the direction of the primary coil) in the animal experiments is equivalent, physically, to a slight increase in the rate of change of the primary current. From the purely physical standpoint, therefore, the minimal stimulus values recorded in each animal experiment should be somewhat smaller than they are. Physiologically, however, this motion of the secondary coil actually decreases the effectiveness of the secondary current as a stimulus for the cortex, and the animal curves accordingly have greater ordinate values than if the secondary coil had been stationary at the separate times that the reactions occurred. This physiological effect of the motion was established by actual experiment. It is supported, also, by the law of stimulation of du Bois-Reymond. Slowly increasing the strength of the induced current is comparable to slowly increasing the physical strength of any other kind of stimulus.

Fortunately, the particular, general level of any curve from a single animal is not important in the present investigation. The general level may be permitted to vary from animal to animal without causing experimental error or any complications of significance, but it is imperative in work of this sort that the rate of change of the primary current and also any equivalent of a rate of change of this current remain constant during each of the experiments with the different animals. The general level alone is influenced by the nature of the primary current.

The features, as well as the general level of a curve, often appear to be influenced by the point of application of the electrodes, by the depth of insertion of the electrodes into the cortical substance and by the depth of the state of anesthesia. Every effort was made, therefore, to keep these factors constant throughout each of the experiments. The electrodes were held in one position by means of a rigid frame which was attached securely to the skull of the animal. The ether bottle was placed in a

large vessel of water, and the temperature of this water was regulated carefully. Ether dripped from a special reservoir into the ether bottle as fast as the anesthetic was used by the animal.

Some special details of the general method here discussed will be stated in connection with the results.

RESULTS. The curves of figures 2, 3 and 4 should be looked upon as skeletons of some of the more instructive protocols obtained from the experimental animals. The ordinates represent millihenrys. The numerical values along the abscissae show the number of single or double observations recorded automatically in each experiment; and, since the observations were recorded at the rate of about one single or double one each minute, the abscissae also indicate the approximate duration, in minutes, of each experiment. When, for convenience, some of the results are not plotted, this will be stated in connection with the curves.

Interpretations of results. It appears plausible that the curve for the right hind leg in figure 2 descended slowly because the opening in the meninges over the cortical area was so small that the cerebral fluid could not escape from the cranial cavity without washing the stimulated region of the cortex. Perhaps the fluid served the dual purpose of transporting nutrient materials and hormones to the cells and of relieving them of accumulated toxic substances. We may presume that when the opening in the dura was so large that the fluid escaped without coming in contact with

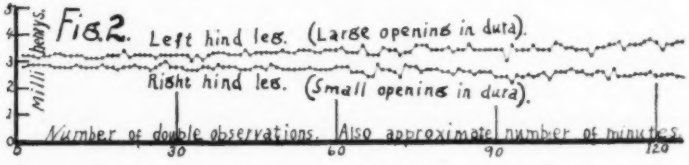


Fig. 2. An opening of about 25 sq. mm. was made in the skull, dura and arachnoid over the principal cortical motor areas in the right cerebral hemisphere. A pair of platinum electrodes was inserted to a depth of about 2 mm. into the cortical area for the left hind leg. Cerebral fluid escaped without coming in contact with the area selected for stimulation. The rise in the curve for the left hind leg indicates an apparent decrease in the irritability for the cortical cells.

The opening in the meninges over the cortical motor area for the right hind leg was so small (a slit about 5 mm. in length) that the escaping cerebral fluid constantly washed the stimulated portion of the cortex. Otherwise, the conditions were the same as for the corresponding area in the right hemisphere.

One hundred fifty-six double observations were made, but the last 31 of these were not plotted. The minimal stimulus values for the left leg underwent a slow increase from about 3.2 to 3.7 millihenrys in about 156 minutes, and the values for the right leg decreased from about 2.8 to 2.3 millihenrys in the same time. The two primary circuits were interrupted at about the same rate (about 62 times per second). The amperage of each primary current was carefully regulated, being kept as nearly as possible at 274 milliamperes.

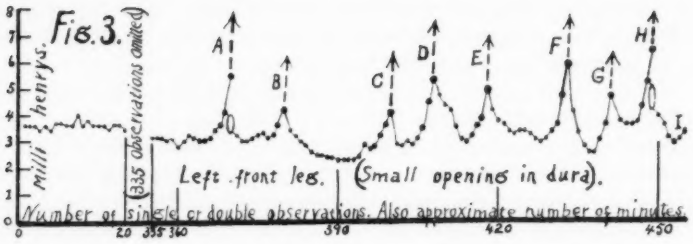


Fig. 3. The area for the left front leg was stimulated for about 518 minutes. Otherwise, the conditions were the same as for the lower curve in figure 2; 335 observations were omitted after the 20 minute interval and 63 results were not plotted after the 455 minute interval. The first part of the curve was very much like the lower curve in figure 2. Large waves finally appeared, the first one being highest at the 369 minute interval. An ellipse in the curve means that the apparatus failed to record a movement that occurred in an unusual direction and that the value of the minimal stimulus was judged to fall at some point within the ellipse. Any point plotted above an ellipse indicates a second movement which occurred and was recorded automatically by the apparatus. The arrows with broken shafts indicate vanishing movements of the leg, and the letters associated with the arrows are the names given to the different types of movement.

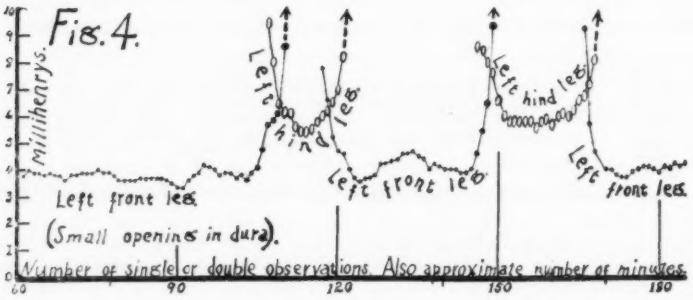


Fig. 4. Two inductoria were used, but one of these was a "dummy" in the sense that it was fed no current and was accordingly not used to stimulate. The pair of platinum electrodes from the secondary coil of the live inductorium were inserted to a depth of about 4 mm. into the cortical substance of the right cerebral hemisphere, the attempt being to strike the cortical area for the left front leg only. The opening in the meninges was a smooth cut about 5 mm. in length. Some preliminary tests showed that a movement of the left hind leg sometimes preceded the reaction of the left front leg. The dummy inductorium was then arranged to record automatically the movements of the left front leg. The live inductorium was not connected with any bodily member, and the secondary coil always continued to fall until it was stopped by hand. Whenever the left hind leg reacted, the position of the pointer on the millimeter scale of the live inductorium was noted. The person who made these readings sat in such a position that the left hind leg and the millimeter scale

could both be seen well at the same time. In view of the facts that the reactions of the left hind leg were not recorded automatically by a mechanical device and that the readings were made while the secondary coil was in motion, the data on this leg are not supposed to be extremely accurate. Each of these observations is accordingly represented in the curve by a small ellipse. The correct ordinate value evidently falls at some point on the long axis of the ellipse.

The results obtained are the minimal stimulus values for the left hind leg and the left front leg. The area stimulated may be spoken of as the cortical motor area for the left hind and front legs. This area is of course more complex than that for either the left hind or front leg, but the movements here recorded are comparable to the pairs of movements A and B, B and C and others in figure 3. In the present case, the secondary coil of the live inductorium moved regularly to within 60 mm. of complete coincidence with the primary coil. The strongest stimulating current had the value, therefore, of about 10.4 millihenrys. The primary current was interrupted at the rate of approximately 66 times per second, and it measured 290 milliamperes. The first 60 and the last 112 results that were recorded are not plotted.

the stimulated cells (fig. 2, curve from left hind leg), the exchange of these materials was not very good and that the minimal stimulus values accordingly increased slowly. The size of the opening in the meninges is evidently a factor which demands special attention in any study of the apparent irritability of cortical cells. This viewpoint increases further in importance when the results of some auxiliary experiments are considered.

The curves invariably rose when the cortical areas were stimulated through the unbroken dura. The rise was especially marked when the electrodes were placed against the dura with considerable pressure. The same occurred, but to a lesser extent, when the electrodes were forced through two very small openings in the dura and were then forced to a distance of a few millimeters into the cortical substance. The presence of the relatively static fluid about a cortical area does not appear, therefore, to favor the exchange of materials as well as does the constant washing of the cells by the fluid as this escapes through the opening in the dura mater.

When the opening in the meninges was so large that the curve tended to rise (fig. 2, upper curve) and pieces of dry filter paper were applied to the cortex in the immediate vicinity of the electrodes, the curve generally changed direction; it often became more horizontal, and it fell slightly in a few cases. Papers soaked in water or physiological saline seemed to cause the curve to rise more slowly in some instances, but there was ordinarily no appreciable effect. Washing the area with physiological saline, by dropping the fluid onto the cortex from a pipette, probably caused the curve to pursue a more horizontal course in a few cases. Washing the area in the same way with cerebral fluid or with serum from the animal usually caused an appreciable fall in the curve.

The dry filter papers evidently served the dual purpose of soaking up the fluid containing the toxic substances and thereby permitting some new

fluid deep in the nervous tissue to take the place of that which passed into the papers. We may presume that this new fluid contained some nutrient material which was utilized by the stimulated cells. Papers soaked in water or physiological saline could not cause an interchange of substances to such a marked extent. Washing the area with physiological saline probably removed some of the toxic substances and accordingly decreased the usual rate of decline of the irritability of the cells. Washing the area with cerebral fluid or with blood serum probably removed some of the toxic substances and also supplied some needed material to the stimulated cells.

The curve in figure 3 is an example of how a relatively smooth curve from a healthy dog may show great fluctuations in the apparent irritability of a cortical area after this is stimulated with minimal currents for a long period of time. Other observations that were made may be presented briefly by referring to the letters placed above the large waves in the curve.

The minimal stimulus for a type of movement, which we may call the A-activity, the A-movement or simply A, increased beyond the threshold stimulus for another type of movement, B. The B-movement occurred in such a direction that it was not recorded the first time by the apparatus which was especially adjusted to record A. The minimal stimulus for this particular reaction of the leg was judged to fall at some point on the long axis of the ellipse at the 370 minute interval. After the secondary had moved closer to the primary coil, the stimulating current was strong enough to elicit the A-movement. As usual, this movement tripped the apparatus and stopped the motion of the secondary coil. The arrow with the broken shaft indicates the belief that A could have been obtained later still with stronger stimuli if these had been applied. By changing the position of a small pulley, over which the thread passed that connected the leg and the lever at the work adder, the apparatus was adequately adjusted to record the new movement whenever it took place. The same statements are applicable to the wave at H, which also contains an ellipse. Even though these two waves are very much alike in the curve, they are the records of different types of movement of the leg.

The minimal stimulus for B increased until another type of movement C, occurred first. The same is true of other pairs, as C and D, D and E and so forth, in which the new activity in any instance was so much like the preceding one that all of the individual reactions were recorded automatically without any readjustment of the apparatus being necessary. The arrows indicate that the vanishing activities could have been followed up and obtained by using stronger stimuli, as was actually done in the case of A and H. On the basis of direct observation of the reacting member, it was concluded that H was merely a reappearance of D, that K (not plotted) was a third appearance of D and that G was a second appearance of B.

However, there is room for doubt concerning the reliability of some of these judgments. The difficulty in making accurate comparisons in such cases lies in the fact that the activities are temporally far apart. It is much easier to observe whether temporally juxtaposed activities are the same or different. These always seem to differ from each other in one or more respects. In fact, any one of the waves in the curve seems to mean that as one activity or type of movement vanishes, another one takes its place.

The curve shows a "Treppe" or "staircase" phenomenon for B and each of the succeeding activities. This is indicated by the descending limb of each of the waves. This feature of the curve seems to be subject to the following translation or interpretation. A cortical area, as that for the B-movement, that gradually weakens, slowly loses its inhibitory effect upon other cortical areas, such as the special area for the C-movement. As this occurs, the minimal stimulus for the C-area decreases gradually in value until the B-area is very weak or is "knocked out" temporarily or permanently. Cortical areas for two activities, as B and C, inhibit mutually when both are excited, and there is accordingly an inverse ratio existing between the strengths of the minimal stimuli for the two areas at any minute. This is the case whether or not both of the areas are sufficiently excited at any minute interval to cause the two separate movements of the leg. A minimal stimulus for the B-area may also affect the C-area without necessarily causing the C-movement, and, at another time, a minimal stimulus for the C-area may also excite the weakened B-area without necessarily causing the B-movement. Therefore, if the weakening of the B-area means, among other possible things, a weakening of its inhibitory power over the C-area, the gradual decrease in the strength of the minimal stimulus required to excite the C-area is a very natural consequence. In summarizing the statements of belief in this paragraph, it may be stated that each wave in the curve means a transition from one activity to another, the ascending limb being due to the weakening of one cortical area and the descending limb being due to the decrease in the inhibitory power of the first upon the second area.

Muscles of the legs of an animal which has been enesthetized for a considerable time show marked staircase effects when stimulated directly with a sub-maximal, induced current at regular, short intervals. The cumulative effect of sub-minimal stimuli is also easy to obtain, but this is of course only a special manifestation of the staircase phenomenon, obtained in this case by applying a very weak stimulus at short intervals. During the time that the injured muscle (a type of injury due to the prolonged anesthesia) is being revived by the repeated stimulation of it (Swindle, 1922), the strength of the minimal stimulus for the cortical motor area for the muscle should be greater than when the muscle is completely revived and accordingly possesses its normal states of irritability and contractility. This

behavior of the muscle, however, does not enable us to explain the fact that one flexor muscle begins to react only when another one weakens, unless there is mutual inhibition of the flexor muscles of the leg. It also fails to account for the observation, which was made in a number of cases, that the muscles react well to the induced current after it is no longer possible to elicit a reaction of them by stimulating the cortex. The muscles appear, therefore, to play only a small part, if any, in the production of the waves.

It may be useful to mention in connection with figure 3 that the troughs of such waves often coincide with somewhat periodic spasms of the animals. Spasms were not perceptible in the dog from which the curve of figure 3 was obtained until near the end of the experiment. It would perhaps be more correct to state that the experiment was brought to a close sooner than was originally intended because the spasms finally became so strong that they interfered seriously with the accurate recording of the reactions of the leg during the troughs of the waves. The first perceptible spasm began at about the 490 minute interval. The earlier spasms were too mild to be perceived directly, but they were recorded with the apparatus; each trough in figure 3 means a mild spasm, and the crest of each wave means the relatively great decrease in the apparent irritability which is characteristically manifested by an animal after each spasm.

The spasms were tonic in this and also in some other dogs. A more frequent type of spasm embodies a number of periodic reactions which are principally clonic in nature and which may be spoken of collectively as epileptiform movements. A single dog may show both clonic and tonic spasms at separate times or there may be a mixture of the two types. In some instances the spasms are somewhat difficult to classify, but they may be indicated with a fair degree of accuracy by saying they embody periodic shivering and closely related activities. The direct observations and recorded results suggest that the cause of any of these spasms is the temporary or permanent elimination of an inhibitory force. If inhibitory forces are eliminated at fairly regular intervals, as is indicated in figure 3, a series of either perceptible or imperceptible spasms is the result. No evidence was obtained that might indicate that a heightened irritability of a cell or group of cells was a factor contributing to the cause of any spasm.

In discussing figure 4, we may speak of one general area for the legs on the left side of the body. This area accordingly consists of two complex sub-areas, one for the front leg and one for the hind leg. These sub-areas may be likened to two of the simpler sub-areas for special groups of muscles of a single leg. In the present case, the electrodes were inserted into the cortical substance at such a point that always one leg and sometimes both legs reacted sooner or later while the strength of the stimulating current in-

creased slowly from about 0.6 to 10.4 millihenrys. For instance, at the 90 minute interval, the left front leg reacted and stopped the secondary coil of the dummy inductorium when the stimulating current measured 3.3 millihenrys. As the current strength continued to increase, from 3.3 to 10.4 millihenrys, the front leg reacted in a number of other ways which were not recorded, but there was no movement of the hind leg. At the 115 minute interval the hind leg alone reacted. The first movement of this leg occurred when the stimulating current measured 5.5 millihenrys. This leg also reacted in some other ways while the current was increasing from 5.5 to 10.4 millihenrys. At the 107 minute interval, both legs reacted, the front one first at 5.7 and the hind one first at 9.4 millihenrys.

The curve indicates mutual inhibition between the two complex sub-areas. Shortly after either of the curves begins to ascend, the other one begins to descend, as is also true in the case of more elementary sub-areas (fig. 3). Any further discussion of figure 4 would be essentially the same as previous discussions of the inverse ratio existing between the values of the minimal stimuli for such pairs of activities as A and B or B and C of figure 3. In the last analysis, the waves in figures 3 and 4 are fundamentally the same.

General discussion. The periodic variations in the curves are probably related to various other phenomena, such as the waves of gastric secretion (Bickel, 1925), the fluctuations in the chronaxie (Tedeschi, 1924), the positive and negative induction of Foursikov (1923), the phenomena of dominant and sub-dominant activities as manifested by normal animals (Swindle, 1915) and man (Swindle, 1916), some slow and rapid changes in the action of the heart (Swindle, 1925), certain kinds of mild and strong spasms, retinal rivalry and the phenomenon commonly known as the fluctuation of the attention. The waves in the curves appear to indicate some outstanding cases of the serial action of different portions of the nervous system. The serial plan appears to be the normal as well as the abnormal plan of action. The fluctuations are only more apparent when many or all of the nervous elements are depressed unequally, as is presumably the case after prolonged activity and during various diseases including the important deficiency disease commonly known as starvation.

The unequal depression appears to be the important factor contributing to an increase in amplitude of the waves. If none of the sub-areas of a general area are depressed and all are equally irritable, there can be no waves to indicate the transition from one activity to another. If all of the sub-areas are equally depressed and all possess the same intrinsic irritability, there can likewise be no waves to indicate the transitions. In either of these instances any sub-area would have to weaken only slightly before another sub-area would begin to react. We may assume that the sub-areas of any general cortical motor area are about equally irritable in

the normal or relatively normal animal and that the waves in the curves are therefore very shallow, as in figure 2, if perceptible at all at the transitions.

CONCLUSIONS

1. When cerebral fluid flows over a cortical motor area which is being excited at the rate of about once each minute with minimal stimuli, the stimulated cells are benefited, presumably because of an exchange of nutrient and toxic substances. The cerebral fluid is the medium of this exchange. This explains the slow decrease in the value of the minimal stimulus required to excite the area when the opening in the meninges is small. When the opening is so large that the fluid escapes from the cranial cavity without washing the stimulated cells, the exchange of materials is poor and the value of the minimal stimulus increases slowly.

2. A cortical motor area for a bodily member consists of several sub-areas which can be "knocked out" one after another by stimulating the area with minimal currents at the rate of about once each minute and for a total time of three hours or more while the electrodes are held rigidly in one position. Sub-areas are relatively easy to knock out if the animal is diseased and emaciated. In some instances, a sub-area which appears to be knocked out may function again.

3. As one sub-area gradually weakens, the current required to excite it must be made gradually stronger. The time must finally arrive, therefore, when the current is strong enough to excite a second sub-area before the minimal current strength for the first one is reached. This is the crucial point in the transition from one activity to another. Each complete wave in a minimal stimulus or threshold curve means a complete transition. Aside from being directly responsible for the ascending limb, the weakening sub-area is indirectly responsible for the descending limb of the wave. As the first sub-area continues to weaken, the second one reacts to weaker and weaker stimuli as the inhibitory force of the weakening sub-area decreases.

4. The troughs of the waves in a minimal stimulus curve mean either incipient or real spasms, a real spasm being one which is apparent upon direct observation of the animal. The crests of the waves mean the state of lethargy or relative inactivity which characteristically follows a spasm.

The greater number of the experiments of this investigation were very long, the longest one lasting about eleven hours. When it is considered that the anesthetic and the primary currents were carefully watched and regulated, that the apparatus was set approximately once each minute so that a single or double observation could be recorded automatically, that it was necessary to tabulate these results and that very extensive proto-

cols were taken in some cases, it becomes evident that the separate experiments were endurance tests for those who took part in the work. I wish to express my thanks to Dr. Harry Beckman, Dr. J. P. Hettwer and Mr. E. H. Payne who volunteered their services in these trying ordeals and then painstakingly criticised the manuscript.

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INCIPIENT SPASMS CAUSED BY APPLYING STRYCHNIN LOCALLY TO CORTICAL MOTOR AREAS

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Some of the more important terms defined in a previous paper on "Slow and Rapid Variations in the Minimal Stimulus Required to Excite Cortical Motor Areas" (THIS JOURNAL, this number, p. 638), and which will also be used in the present paper, are the minimal stimulus curve, the imperceptible or incipient spasm and the perceptible or real spasm. It will be well to re-state the meanings of these terms briefly before considering the present series of experiments.

When the values of the minimal induced current required to excite a cortical motor area at regular intervals are expressed in terms of a convenient unit of mutual inductance, the millihenry, and plotted as ordinates with the number of successive determinations as abscissae, the resulting curve is the minimal stimulus curve for the area. Any fall in this curve means an increase in the apparent irritability of the area. The test of excitation of the cortical cells is a movement of the appropriate bodily member, such as a hind leg. If the cortical area is excited at one minute intervals with minimal currents, large waves eventually appear, gradually or suddenly, in the minimal stimulus curve. The troughs of the waves mean either incipient or real spasms, and the crests mean the type of lethargy or relative inactivity which characteristically intervenes between spasms. Incipient as well as real spasms may be limited to a single leg of an animal, and the real spasms may be either clonic or tonic in nature. The reason for identifying the troughs of the waves (periodic increases in the apparent irritability of a cortical area) with incipient spasms is that the troughs can be observed to coincide with real spasms if the depth of anesthesia is sufficiently decreased after the waves are well developed.

If the experimental animal is diseased and emaciated, large waves may appear in the curve after thirty or forty minutes of stimulation. On the other hand, if the animal is healthy and well nourished, the cortical area can be stimulated for three hours or more before the large waves develop. Now strychnin applied directly to a cortical motor area alters the minimal stimulus curve in certain interesting ways. Of chief interest is its tendency

to hasten the appearance of the large waves in the curve and later to convert the incipient spasms into real ones even though the depth of anesthesia remains constant. The strychnin spasms, as well as those obtained without strychnin, may be limited to a single leg and may be clonic or tonic in nature. Essentially, therefore, strychnin hastens and augments certain effects which can be obtained from an animal by merely stimulating a cortical motor area repeatedly with minimal currents.

Particular attention was given to the incipient strychnin spasms because they were considered to be relatively simple and yet fundamentally the same as the more intense or real spasms. The experimental animals were thirty-two dogs, seven cats and five rabbits. This number includes the animals used in certain control experiments but does not include five or six dogs from which the results could not be considered reliable because of bad technique or unavoidable interruptions in the work.

METHODS. It proved to be an unsafe method of procedure to secure first a normal or control curve by stimulating a cortical motor area at regular intervals for a considerable time, to apply a strychnin solution to the area and then to consider that some or all of the variations occurring in the curve after the application of the solution are caused by the strychnin. This procedure was used for a time but was frustrated by a few instances in which spectacular variations made their appearance in the curve just before the set time had arrived to apply the strychnin. In order to avoid the difficulty, corresponding cortical motor areas of the two cerebral hemispheres were stimulated in the same way throughout any experiment and strychnin was applied to only one of them. The selected areas were usually those for the hind legs. This method necessitated the use of two like pieces of apparatus for stimulating the areas and recording automatically the strengths of the minimal currents required to elicit the reactions of the legs. The curve obtained from the left area and the right hind leg was the control curve in most cases. That from the right cortical area and the left hind leg was generally the strychnin curve. An experiment was considered successful only when the control curve remained relatively smooth. Since the apparatus here used was described at length in the paper already referred to, only a brief description of it will be given at this time.

The apparatus for each of the cortical motor areas consisted essentially of a Porter inductorium so arranged that the primary approached the secondary coil at the rate of approximately two millimeters per second until the leg reacted. This apparatus was further arranged so that the movement of the leg suddenly and automatically stopped the motion of the secondary coil. The extent of relative superposition of the coils was then determined and translated into millihenrys by referring to a curve of mutual inductance previously obtained for the Porter inductorium. A

double reading, one for each hind leg, was made at the rate of about once each minute. The apparatus proved to be very reliable and useful, especially because it could be adjusted to record a movement occurring in one direction only. In many instances it selectively recorded a movement in a specific direction although this movement was preceded by others during the same minute interval. It is true that the selective action of the apparatus was not shown in some cases, but this can be attributed to the circumstance that the differences in direction of the different kinds of movements were too nearly the same.

Strychnin solutions of various strengths were used, and different methods were employed to apply them. A solution was dropped on the area at certain intervals; small pieces of filter paper were soaked in a solution and placed one at a time on the area, the papers being renewed at regular intervals; however, the strychnin was generally applied with a hypodermic syringe by injecting a solution of the sulphate into the cortical substance near the stimulating electrodes. The last of these methods was risky but very good when the injection was properly made. It was risky because the strychnin sometimes passed quickly into the circulation and caused marked systemic as well as local effects. Whenever the control curve showed marked systemic effects it was considered that a blunder was made in injecting the solution, that a cerebral vessel was probably broken or pierced with the needle. In spite of the risk, however, the method was desirable because the local effects appeared very soon after the injection. That the early appearance of the strychnin effects is quite important can be realized readily if reference is again made to the fact that repeated stimulation of an area eventually causes great variations in the minimal stimulus curve. If the local action takes place quickly it is often possible to finish an experiment on a healthy, well-nourished animal before such irregularities appear in the control curve.

A further advantage of the injection method is that the opening in the meninges need be only large enough to insert the electrodes and hypodermic needle into the cortical substance. Absorption of the strychnin probably takes place less rapidly when the opening is small than when a large portion of the meninges is injured.

Two separate openings were usually made over each of the chosen areas. These openings were a slit about five millimeters in length for the insertion of the electrodes and a very small opening nearby for the easy insertion of the hypodermic needle. The injection of strychnin into one area was generally accompanied by an injection of the same amount of physiological saline into the corresponding area of the other hemisphere. The needle was inserted carefully through the small opening previously made for it and to a depth of a few millimeters into the cortical substance. The solution was then injected slowly as the needle was gradually withdrawn.

Special precautions were taken to avoid absorption of the strychnin from the injured bone and scalp. The cut edges of the bone and the wound in the scalp were carefully covered with a moderately hard wax.

RESULTS. The minimal stimulus curves presented in figures 1 and 2 were obtained from healthy, well-nourished, adult dogs. Both figures are presented because the strychnin curves for the left hind legs are the extremes of the strychnin effects that were obtained under the apparently identical conditions subsequently stated. These curves have certain general features in common. The relatively rapid waves are very similar and both curves rise for thirty minutes or more before falling to relatively low levels. There are marked differences, however, in the rates of the initial rises and the later falls in the curves. It may also be observed that the general rise near the end of the curve in figure 1 is absent in the corresponding curve of figure 2. Although such differences exist between the strychnin curves, the control curves are practically identical.

Interpretation of the results. The curve for the right hind leg (fig. 1) is the control curve up to the 108th minute interval. It fell in a short time from about 3.8 to 3.2 millihenrys. One probable cause of the fall was the small opening in the meninges, the other was the systemic action of the strychnin absorbed from the opposite side of the brain. As the cerebral fluid escaped through the small opening, it washed the stimulated cells and presumably carried some nutrient materials to these cells and relieved them of some toxic substances. We may imagine that the cells were benefitted to such an extent by this exchange that the minimal current finally required to excite them was weaker than at the start. The fall here observed is perhaps more rapid than any which occurred in other experiments when the opening was likewise small but no strychnin was used. The general action of the absorbed strychnin is probably a factor which contributed slightly to the fall in the curve. The same statements are also applicable to the control curve in figure 2.

The strychnin curve (for the left hind leg, fig. 1) is an extreme case of an increase in the apparent irritability of a cortical motor area, but even in this unusual case there is an appreciable rise in the curve before the general fall begins. Strychnin solutions strong enough to cause unmistakable effects also caused the initial rise and often the final fall in the minimal stimulus curve. In several curves, as in that for the left hind leg in figure 2, the rise was a very spectacular feature. Sometimes there was no fall below the normal values. The latter part of the curve for the right hind leg (fig. 1 or 2) is a specific instance of this, although it is scarcely fair to cite such a case without mentioning that similar results were obtained from some animals, especially diseased and emaciated dogs, which had been under ether for only a short time and which had not had strychnin before.

It is quite apparent, therefore, that the results of this investigation do not agree entirely with either variety of results reported by earlier workers. One of these varieties indicates an increase only and the other a decrease

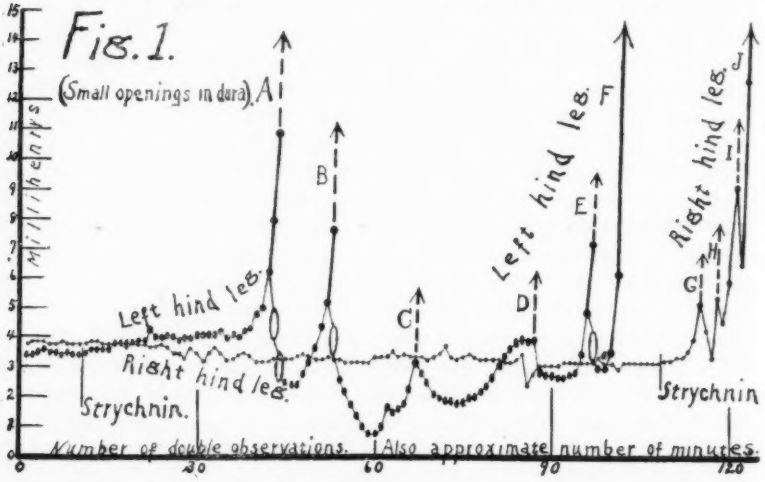

Fig. 1. Smooth cuts about 5 mm. in length were made in the meninges over the corresponding cortical motor areas, in the two cerebral hemispheres, for the hind legs. These areas were stimulated with two separate inductoria. The platinum electrodes were inserted to a depth of about 4 mm. into each area. A second opening just large enough for the easy insertion of a small hypodermic needle was made in the dura immediately above each pair of electrodes. The edges of the cut bone and the scalp wound were covered securely with wax. After ten observations were recorded, the hypodermic needle was inserted through the small opening near the electrodes in the right cortical area, its point was forced slowly to a depth of about 10 mm. and then 5.0 c.mm. of a 1.0 per cent solution of strychnin sulphate were injected as the needle was withdrawn slowly. An equal amount of a 0.9 per cent solution of NaCl was then injected in the same way into the corresponding area of the other hemisphere. The rates of interruption of the primary currents were about the same, between 69 and 70 per second. Each primary current measured approximately 270 milliamperes.

The arrows with broken shafts indicate vanishing movements of the leg. The arrows with unbroken shafts mean the movements were followed up and obtained by using sufficiently strong currents. An ellipse in the curve means that the apparatus failed to record a movement which occurred in an unusual direction and that the value of the minimal stimulus for this movement was judged to fall at some point on the long axis of the ellipse. Any point plotted above an ellipse indicates a later movement which was recorded automatically by the apparatus. Only two other values could be obtained for the special activity indicated by the letter F. These are 32.0 and 41.1 millihenrys. The values obtained but not plotted for J and at least one later activity are 17.9, 36.0, 55.5 and 62.4 mh. The dog was killed many minutes after the experiment was finished.

only in the irritability of cortical motor areas to which strychnin solutions are applied. A short but useful review of some of the more interesting of these investigations is given by Poulsson (1920).

The initial rise in the strychnin curve is probably due to a general depressing action of the strychnin. The general fall in the curve that sometimes takes place relatively late in the strychnin experiments is probably due to a further depression of the cortical cells by the locally applied strychnin. In other words, the strychnin exerts a depressing influence only upon the protoplasm of the cells. When first applied, it depresses all of the cells about equally, but there is a limit to this particular

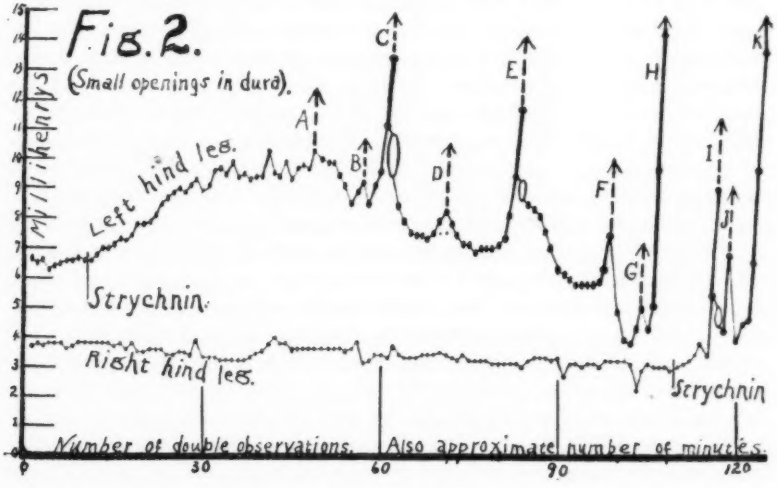

Fig. 2. The conditions appeared to be the same in every respect as for figure 1. One more value, 28.2 mh., was recorded for H, and two more, 18.6 and 41.3 mh., were recorded for K. The dog was killed many minutes after the experiment was finished.

action. Any further depression that occurs takes place only as the cells become altered chemically due to their activity and consequent catabolic changes associated with fatigue; then they may be "poisoned out" completely by the strychnin. If all the cells of a cortical motor area should react together—an improbable situation except under extraordinary conditions—all would be poisoned out together and the curve would rise rapidly. If the general area should respond in the particular fashion that means various sub-areas or portions of it react in a serial order, the sub-areas should be poisoned out in the particular order in which they react. It seems probable that a general fall in a strychnin curve is only an indication that some groups of nerve cells which are depressed but can still react are

no longer inhibited by other portions of the cortical area that have been poisoned out by the strychnin. This would lower the value of the minimal current for the portions or sub-areas of the general cortical area that can still function. In studying the poisoning-out process still further it will be convenient to refer to the letters associated with the arrows in the figures. Figure 1 may be selected for this discussion.

A is the name for a particular type of movement of the leg. The individual A-movements occurred in a particular direction. We may assume that a sub-area, the A-area, of the general cortical motor area for the leg reacted repeatedly and caused the individual reactions of a particular group of flexor muscles, the A-group. The A-area weakened gradually until the minimal stimulus for it was stronger than that required to excite another sub-area, the B-area, and cause the reaction of the B-group of flexor muscles. The leg now moved in a new direction because of the B-activity and accordingly did not trip the apparatus which was especially adjusted to record the A-movements automatically. The secondary coil of the inductorium continued to fall, therefore, after the B-movement occurred and, even though certain other movements followed this B-movement, the motion of the coil did not cease until the current reached the proper strength to elicit an A-movement by exciting the weakening A-area. The minimal stimulus value for the first of the B-movements was judged to fall at some point on the long axis of the first ellipse drawn in the curve. The second ellipse and the value plotted directly above it indicate that a similar series of events happened once more. Before the third B-movement was elicited the apparatus was carefully adjusted to record the individual B-movements until the B-type of movement or activity disappeared as the B-area weakened. The arrow with the broken shaft at A indicates the belief that if the apparatus had not been adjusted to record the B-movements, some A-movements would have been recorded at still later minute intervals.

As the A-activity gradually vanished, it was paradoxically preceded and succeeded by the B-activity. The apparent contradiction in the use of these terms arises from the fact that at any minute interval the current causes both movements at separate times and that a few minutes after one activity has become so weak that it is negligible the other activity is strong. The same paradoxical situation exists, either actually or theoretically, with respect to all the other pairs of activities such as B and C or G and H. There is no paradox associated with the unpaired F-activity. Any of the paradoxes means a transition from one activity to another. Each transition means a branching or splitting of the curve. Each split in the curve means that the minimal current for one sub-area gradually decreased in value as the value of the minimal current for another sub-area

gradually increased. The gradual increase in strength of the minimal stimulus for a sub-area means that the inhibitory force associated with this sub-area also decreased gradually and permitted the strength of the minimal stimulus for another sub-area to undergo a gradual decrease in strength. The weakening of the sub-areas in these experiments is attributed to the action of the locally applied strychnin. Indirectly, therefore, the strychnin is also responsible for the descending limb of each wave in the curve. Strychnin, by virtue of its property of reacting with and depressing especially the active nerve cells, is the cause of the general fall in the curve, the deep troughs and accordingly the incipient spasms. The periodicity of the troughs means incipient spasms at regular intervals. The incipient spasms may become perceptible or real spasms if a large amount of strychnin is applied or if the depth of anesthesia is sufficiently decreased after a moderate amount of strychnin is applied locally to the area. These spasms continue until the last of the sub-areas is poisoned out. In the case of F, the sub-area weakened and none was left to function in its place.

It may be useful to discuss briefly one or two auxiliary experiments which show definitely the relationship which exists between the phenomena referred to as incipient and real spasms. One dog was only mildly anesthetized throughout the experiment. A large crystal of strychnin sulphate was placed on the cortical area for the left hind leg. The minimal stimulus curve for this leg rose gradually for about twenty-five minutes before it fell and large waves developed in it. During the greater number of the troughs, the animal appeared to recover from the ether to a slight extent and show fairly strong spasms which were limited to the left side, the hind leg being affected considerably more than the front one. Extension instead of flexion of the leg occurred at some of the troughs and interfered with the automatic recording of the movements. In the latter part of the experiment, the cortex was not excited with the electric current and the spasms continued to occur periodically. A part of this experiment was repeated on another dog under somewhat different conditions. The trephine opening was made in the skull while the animal was anesthetized with ether. After the dog had fully recovered from the effects of the anesthetic and was running about in a room, a large crystal of strychnin sulphate was placed as accurately as possible on the cortical motor area for the left hind leg. Spasms of the left legs soon occurred at fairly regular intervals. The left hind leg was affected more than the left front one. The entire body was later in spasms.

Under certain conditions, as after long exposure or prolonged excitation of a general cortical motor area, all of the sub-areas may be in such a chemical state that strychnin can rapidly depress all of them. This

would account for the general and rapid rise in a minimal stimulus curve as that at the end of the curve for the right hind leg in figure 1 or 2. A diseased or emaciated condition of an animal might have a similar effect.

It seems safe to assume that all of the sub-areas for the right hind leg, figure 1 or 2, were in such a chemical state at the time the strychnin was injected that the general, rapid rise occurred in the curve. The existence of the waves shows that here, too, the strychnin acted the more strongly on the active sub-areas and poisoned them out in the order in which they became active. The relatively brief duration of the troughs might indicate that although activity of the sub-areas favored the selective action of the strychnin, only a short period of activity was essential in any case for a sub-area to be poisoned out.

GENERAL DISCUSSION. The results reported show that the waves in the minimal stimulus curves are so closely related to the periodic spasms in typical cases of strychnin poisoning that a discussion of one should also suffice for the other phenomenon. The periodic troughs in the strychnin curves are the graphic representations of incipient or mild spasms if only small or moderate amounts of strychnin are applied when the anesthesia is profound, and they represent spectacular or real spasms if the amount of strychnin is large and the anesthesia is profound or if the amount of strychnin is relatively small and the anesthesia is light or absent. We may judge from this that the intense strychnin spasms either occur spontaneously (i.e., the stimulus is not definitely known) or can be initiated very easily by stimulating the poisoned animal in certain ways at the regular intervals at which the troughs occur. In other words, the poisoning out of some portion of the nervous system removes an inhibition from another portion which now reacts and causes a spasm if the proper stimulus is applied. After this second portion reacts long enough for its chemical nature to change sufficiently for the strychnin to act on and depress it, it in turn is poisoned out. A third portion then reacts with still less inhibition, and so on. Soon the complex respiratory center, which is of course subject to the poisoning-out process, is completely poisoned out and the animal dies of suffocation, or it eventually lies in a profound state of narcosis if artificial respiration is given. During this state of strychnin narcosis, the nervous system is so inactive that the strychnin present in the body does not attack the nerve cells to any considerable extent, and the entire nervous system recovers somewhat from the previous strychnin action. While this recuperation is going on, the strychnin is also being made harmless by being excreted and perhaps also by one or more other processes.

In the course of the recuperative period, the nervous system reaches the stage at which some portion can respond fairly well to external stimuli, i.e., the animal shows signs of recovery from the strychnin narcosis. As

this portion responds it is attacked and depressed by some of the strychnin which is still potent. Then another portion reacts strongly because it is not inhibited by the first and the result is a mild spasm. This second portion is attacked while active and is poisoned out. Then a third portion reacts with still less inhibition and the result is a stronger spasm. The spasms of this series eventually become weaker and disappear only as soon as the strychnin is rendered sufficiently harmless that there is no longer an appreciable selective action for any active portion of the nervous system.

When we are concerned with an entire animal in contrast with a cortical motor area and a leg, it may not be possible to speak very definitely of the portions of the nervous system that are poisoned out in the serial order, but there appear to be ample reasons for considering a portion of the nervous system at a time. One special reason is the fact that animals seem to do primarily one thing at a time. A more important reason is the orderly appearance and disappearance of the various activities in figures 1 and 2.

The notion that a drug may act selectively on the active sub-areas is related to the idea (Hirschfelder, 1918) that a drug may act selectively on pathological structures. A consideration of the facts known about fatigue leads to the belief that a structure in activity and at rest is in two different chemical states, although it may not be justifiable in all cases to say the activity means a pathological state of the structure. The chemical difference, however, may be responsible for the selective action of certain drugs, such as strychnin, but not necessarily for certain other drugs, such as cocain. Cocain depresses and poisons out the cells of a cortical motor area, but there is a fundamental difference between the local action of cocain and strychnin. Cocain depresses the active and inactive sub-areas with about equal rapidity and accordingly causes only a general rise of the minimal stimulus curve; exceptionally, it causes periodic waves in the curve. Strychnin, on the other hand, exerts a powerful depressing influence on the active sub-areas and depresses the inactive ones only mildly. It accordingly causes first a general rise and then a number of periodic waves during a general fall in the minimal stimulus curve; exceptionally, the general fall does not occur.

The selective action of strychnin reminds one of the effect of an electric current applied repeatedly to a cortical motor area. The current causes a sub-area to react until it weakens because of fatigue. It may be said that the current "knocks out" the sub-area either temporarily or permanently, usually only temporarily. Strychnin seizes upon a sub-area which is active because of the electric current or some other type of stimulus and hastens the weakening process. In both cases the area is first set in activity by some form of stimulus. Then the electric current can selec-

tively (selectively because only the one sub-area is active, the others being inhibited by this one) stimulate and accordingly depress or weaken this sub-area, the strychnin can selectively depress and weaken without stimulating it or the current and strychnin together can cause the area to weaken more rapidly than either the current or the strychnin alone can do it. It is important to recall that a strong current soon depresses nerve and that the minimal strength of current used in these experiments is much greater than that required when the anesthesia is light or absent. It is possible, therefore, that the strengths of current here used were such that the cells were injured and made more susceptible to depression by the strychnin, later applications of the current or by both the current and the strychnin.

If the end-results of strychnin action and repeated applications of the electric current are essentially the same and differ in degree only, it might be presumed that strychnin, like the current, eventually depresses the cells by exciting and fatiguing them. However, it seems as if the various features of the minimal stimulus curves that were influenced by strychnin can not be satisfactorily explained on the assumption that strychnin excites the cells. The variations in the curves also furnish no very good reason for assuming that strychnin increases the irritability of the protoplasm of any of the cells. A more useful assumption appears to be that strychnin depresses the cell protoplasm, especially that of the active cells, and is responsible for the fact that the minimal stimulus values for certain portions of the general area decrease sooner or later as some of the sub-areas are poisoned out and some inhibitory forces are thereby eliminated.

The action of strychnin, as here depicted, is in a way comparable to the action of atropin in increasing the activity of the heart by eliminating the inhibitory power of certain nerve fibers. To assume that atropin increases the heart action by increasing the irritability of the protoplasm of some nerve cells would correspond to the view, which is here rejected, that strychnin causes spasms by increasing the irritability of the individual nerve cells. Some special reasons for making the assumption that strychnin depresses protoplasm are the results of certain earlier investigations. Biberfeld (1901) and Filehne (1901) observed that strychnin solutions strong enough to cause any effect at all, when applied locally to the tongue of the human or to the skin of the frog, cause a decrease only in the sensitivity; Kriz (1924) and Swindle and Kriz (1924) found that strychnin causes depression only, when solutions of various strengths are applied to certain unicellular forms; and Swindle (1925) concluded that strychnin enlarges the color zones and increases visual reliability by depressing the nervous elements concerned in vision.

CONCLUSIONS

1. A cortical motor area consists of several sub-areas which can be "knocked out" or "stimulated out" in a serial order by applying minimal induced currents to the general area at one-minute intervals while the animal is in a state of ether anesthesia. Any sub-area weakens gradually, as is indicated by the gradual increase in strength of the minimal current required to excite it. The graphic representation of this in the minimal stimulus curve is the ascending limb of one of the relatively short waves. In view of the circumstance that the weakening of a sub-area means the weakening of an inhibition, it is easy to understand why the sub-area which next functions reacts to weaker and weaker stimuli until it, too, begins to weaken. In the course of a few minutes the weakening inhibition may be so nearly eliminated that the curve falls to an unusually low level shortly before the weakening of the second sub-area becomes apparent. This low level, which is the lowest portion of a trough between two waves in the curve, is the graphic representation of an imperceptible or incipient spasm. It may be a real or perceptible spasm instead if the anesthesia is light or absent. As a result of the repeated weakening of cerebral inhibitions, spasms may occur at fairly regular intervals. Also, the mean values of the minimal stimuli may become somewhat smaller than the normal ones.

2. If strychnin is applied locally to the general cortical motor area, it depresses principally the active sub-areas after each of these has been active for a certain minimal time. The strychnin accordingly hastens and augments the knocking out process associated with the repeated stimulation. Strychnin depresses the inactive sub-areas only mildly but sufficiently to cause the general rise in the earlier part of the strychnin curve. The complete "poisoning out" of some of the sub-areas and consequently the complete elimination of as many cerebral inhibitions often permits the remaining sub-areas to react in the presence of so little inhibition that the curve falls to a relatively low level. In some instances, even the crests of some of the waves fall below the normal level of the curve.

I am indebted to Dr. Harry Beckman, Dr. Joseph P. Hettwer and Mr. Eugene H. Payne for assistance in these experiments.

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THE CONTENT OF LACTIC ACID AND THE DEVELOPMENT OF TENSION IN CARDIAC MUSCLE

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The discontinuous or rhythmic character of the contraction of cardiac muscle raises a number of questions not ordinarily dealt with in discussions of the physiology of skeletal muscle. The rather elaborate collection of facts and theories concerning the chemistry and mechanics of muscular contraction which have come to the fore in the two decades which have followed the investigations of Fletcher and Hopkins (1907) have been derived chiefly from the study of skeletal muscle and it is largely by inference that our present conceptions may be applied to the heart. The general problem which will concern us in this and following papers deals with the factors which determine the extent of the chemical changes which occur at each systole and lead to the output of energy and the development of mechanical effects. How are the views which have been attained by a study of skeletal muscle, brought to complete fatigue by continuous excitation, to be fitted quantitatively into the picture of maintained rhythmic activity on the part of the heart?

In the case of skeletal muscle we know that the energy of contraction is set free by processes which do not depend upon the presence of oxygen. In the recovery of the fatigued muscle, on the other hand, energy is made available for subsequent activity by the oxidation of certain substances which constitute the ultimate energy reserve. In cardiac muscle, as was clearly indicated by Mines (1913), we conceive these two sets of processes to be going on hand in hand during recurrent systoles and diastoles. How is the steady state of cardiac activity to be analyzed in terms of the magnitude of the opposing factors which are thus brought into equilibrium?

In approaching this analysis, since it is essentially a dynamic problem, it has seemed desirable to employ a method which would give precise information concerning the state of the muscle from moment to moment when the conditions underlying the equilibrium state are changed. For this purpose the measurement of the tension developed in the "isometric" response of a strip of cardiac muscle has proved very suitable, and has led to certain definite and interesting conclusions concerning the anaerobic factor in the equilibrium which will be described in a forthcoming paper

by Redfield and Edsall. We are interested, however, in the chemical equilibria which underlie the state of the muscle and which reflect themselves in its capacity to develop tension. The most important and characteristic chemical change known to occur in skeletal muscle in its activity is the production of lactic acid, and this substance has also been shown to appear in cardiac muscle in similar circumstances (Katz and Long, 1925). The present paper is an attempt to discover what relation may exist between the ability of the muscle to develop tension and its content of lactic acid.

The measurement of tension We used for this investigation strips of muscle cut from the ventricles of the snapping turtle, *Chelhydra serpentina*. The animals were collected about the first of May and were employed within three or four weeks of this time at most. This species was selected because its large size enabled several pieces to be cut from a single heart. The strips were prepared by cutting the ventricle into its dorsal and ventral halves, and in the case of the larger specimens these were each further subdivided into two strips running transversely to the original axis of the heart. These strips were kept until ready to use in oxygenated Ringer's solution made alkaline by the addition of sodium bicarbonate to the concentration of 6×10^{-3} mols. The strips of muscle were then suspended by means of stout linen thread in a glass vessel between a fixed platinum ring and the arm of a spring dynamometer. The latter was constructed by supporting a hack-saw blade in the frame of a jeweler's hack saw. A needle, soldered at right angles to the middle of the blade, served as an arm to which the muscle was attached. A small galvanometer mirror was glued to the blade at the same point and served to reflect a beam of light from a carbon arc lamp on to a centimeter scale. Because of the slowness of the contraction the deflection could be read directly without the aid of a photographic record. For the same reason the system, which had a high natural period of vibration, recorded changes in tension developed by the ventricular muscle with almost perfect efficiency. With a projection distance of 75 cm. and the muscle attached 1 cm. from the spring, a deflection of 10 cm.—corresponding to a tension of 33.3 grams—was produced when the muscle shortened 0.6 mm. With a muscle 3.0 cm. long this involves a 2 per cent departure from perfect isometricity. The proper basis for comparing different muscles is given by reducing the measurement of the total tension developed to the tension per unit cross section, which gives the *stress* set up within the muscle as the result of the contractile process. This is conveniently obtained by the product:

$$\frac{\text{Tension} \times \text{length}}{\text{Mass}}$$

The muscles were stretched to an initial tension of about 3 to 6 grams—for which amount the final tensions developed were corrected. The length of the muscle between the knots in the supporting threads was measured at the initial tension and its weight taken by cutting away the portion beyond these ligatures. The latter, however, was not done until the muscles had been frozen in preparation for the lactic acid analysis.

Inasmuch as the relation between the tension developed by a muscle and its lactic acid content may be expected to depend on a variety of factors; notably, temperature, the chemical nature of the surrounding medium, and the character of stimulation, these were held as constant as could be from one experiment to another (Meyerhof, 1920). All the experiments were done at room temperature which varied between 21.5°C. and 24°C. The Ringer's solution which surrounded the preparations was invariably withdrawn—in order to prevent the loss of lactic acid into it—and the muscles, surrounded by an atmosphere of moist gas, were stimulated rhythmically until they had reached a steady state before the final measurements were made. Stimulation was effected by break shocks from a rotary interrupter, led into the muscles through the platinum ring in the bottom of the muscle chamber and through a fine copper wire tied into the ligature at the upper end. The stimuli were applied at intervals of 5.6 seconds, producing thus a rate of 10.7 beats per minute. Such a slow rate was selected because it was believed that larger yields of lactic acid might thus be obtained than would be the case at higher rates. The only condition which was deliberately varied was the supply of oxygen. By replacing the oxygen in the chamber with a stream of nitrogen, after the Ringer's solution had been withdrawn, the tension developed by the muscles at each successive beat fell off according to a characteristic fatigue curve. When the tension had been reduced to various degrees, the muscles were killed by rapid freezing and the lactic acid content measured. In this way data were obtained which show a certain correlation between this measurement and the degree of fatigue as indicated by the magnitude of the last recorded contraction.

The estimation of lactic acid. The muscles were killed by dropping them into a weighing bottle immersed in salted ice. When frozen solid, the portion of the muscle beyond the ligatures was cut away and the remainder was weighed, and then ground in chilled hydrochloric acid and extracted with the aid of mercuric chloride exactly as described by Meyerhof (1924). After the removal of sugar with copper sulfate and calcium hydroxide, lactic acid was estimated by the first of the two methods described by Clausen (1922). The iodine solution used in the final titration was checked each day against a standard solution of arsenious acid.

Concerning the efficacy of this method Clausen claims that 8 per cent of the lactic acid is lost in the aeration process. The yield depends con-

siderably on the exact conditions under which the process is carried out and we have been at great pains to determine just how it should be done to secure maximal and uniform results. In a series of twenty-five determinations of known solutions of zinc lactate the mean yield was 91.9 per cent. Twelve determinations fell within 1 per cent of this value, four more within 2 per cent, while only four deviated from this mean by more than 3 per cent. Meyerhof considers that an additional deficit of from 5 to 7 per cent is occasioned by an unequal division of the lactic acid between the

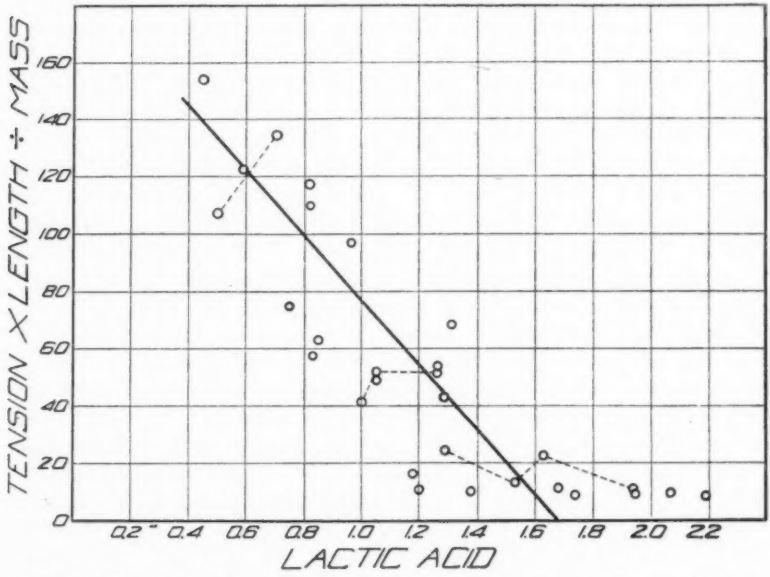

Fig. 1. Correlation between lactic acid content of cardiac muscle and ability to develop stress. Abscissa measures lactic acid concentration in milligrams per gram of muscle. Ordinate measures stress developed per beat in grams per square centimeter. The dotted lines connect data obtained from different strips of the same heart.

extracting solution and the precipitated protein. We have not redetermined this. Since these losses are considered to be proportional to the total lactic acid content, they may be neglected for the purpose of establishing the correlation in which we are interested. We have expressed our results, therefore, as the quantity of lactic acid equivalent to the *uncorrected yield* of aldehyde-producing substances obtained per gram of muscle. In order to test the uniformity of the method of extraction in our hands, one of the large muscles which retract the neck of *Pseudemys concinna* was exposed for 22 hours to chloroform vapor, then divided into

three portions, each of which was extracted separately. The resulting extracts were divided into two portions prior to the treatment with copper sulfate and calcium hydroxide. The yields obtained were 3.45, 3.51, 3.35, 3.43, 3.37, 3.27 mgm. per gram of muscle.

It had been our hope in commencing this investigation to establish the relation between the lactic acid content of the muscle and its ability to develop tension upon a series of strips cut from a single heart. It was for this reason that the largest available species of turtle was selected. It proved, however, that the collective sources of error were so great that a statistical treatment of a large number of measurements was necessary in order to give a significant result. We have, consequently, employed strips cut from the hearts of eleven different specimens of *Chelhydra serpentina*, aggregating thirty determinations of the lactic acid content and the magnitude of the stress set up in the muscle simultaneously. Two of these determinations were rejected because of known analytical errors. The others are shown in figure 1. These data leave no doubt that the ability of the strips of cardiac muscle to develop tension is closely correlated to their lactic acid content.[1] The points scatter rather widely about a straight line. It might be suspected that the scattering is due to individual differences in the various hearts from which the strips were cut. While this is undoubtedly somewhat the case, certain experiments show that the full degree of scattering occurs in a series of strips cut from a single heart. This fact has been brought out in the figure by connecting certain of the data obtained on strips from the same heart by dotted lines. The scattering is to be attributed in part to the large number of measurements (tension, length, weight, and lactic acid content, most of which are obtained by the difference of two readings) upon which the position of each point depends. The chief cause of divergence is, we suspect, differences occasioned in cutting the ventricular strips. The fibers of the ventricle have a most complex arrangement, and in consequence no muscle could be more ill-adapted to our purpose than it is. While the superficial fibers seem to be arranged in bundles most of which are transverse in their direction, the fibers in the internal mass of muscle are not so arranged and must be damaged by cutting in such a way that they contribute little to the development of tension while making up a very significant fraction of the weight of the preparation. These conditions must vary greatly from one strip to another.

[1] During the preceding January we made a series of twelve measurements upon strips of the heart of *Pseudemys concinna* which showed a similar correlation between the development of stress and the lactic acid content of the muscle. The numerical relation was, however, somewhat different. Because of certain subsequently discovered sources of analytical error we do not present these data.

The straight line about which the points fall may be represented by the equation

$$S_b = K\,(L_m - L_b)$$

where S_b is the stress developed in the contracting strip at any beat, L_b is the lactic acid concentration at the time of that beat, L_m is the maximum lactic acid concentration of complete fatigue, and K is a constant. The value of L_m is 1.75 mgm. per gram and that of K, 115. This equation may be taken as a first approximation to represent the relationship in question. In using it, it must be borne in mind that it is merely this, that there is every possibility that some other function may truly represent the facts; that there are no data representing the state of affairs when very little lactic acid is present; and that the data actually suggest a departure at very great degrees of fatigue. With these reservations it leaves us with a very much more definite idea of the relation of lactic acid to the fatigue of cardiac muscle than we previously possessed, and should make it proper to draw some conclusions with regard to the chemical condition of cardiac muscle from the development of tension.

Certain quantitative aspects of these results are also of interest. Katz and Long (1925) and Hines, Katz and Long (1925) point out that the lactic acid content of the cat's heart driven to exhaustion, in heat rigor, and in caffeine rigor, is much less than that of the skeletal muscle of the same animal. While we have not determined the fatigue maximum of skeletal muscles of *Chelhydra serpentina*, it is noteworthy that the highest lactic acid yields we obtained (1.2 to 2.2 mgm. per gram) are considerably lower than the corresponding figures usually observed with amphibian skeletal muscle (Fletcher and Hopkins, 1907; Meyerhof, 1921). We do not believe these low values are due to failure of the conducting mechanism before the contractile process is exhausted, for such failure, which sometimes occurs, is signalized by a clearly recognizable tendency to alternation. When this occurred the experiment was terminated at once, but, as may be seen from the data in figure 1, the contractile power of the muscle in many cases was reduced very greatly before it became necessary to end the experiment. In this connection it is interesting to note that Evans (1925) finds even lower values to characterize smooth muscle.

In our experience it was noteworthy that we could never reduce the lactic acid content of a ventricular strip which was being driven rhythmically at the rate of 10.7 beats per minute to less than 0.4 mgm. per gram of muscle. Frequently muscles contained 1.0 to 1.2 mgm. or more under the best conditions we could establish. Working with unfatigued mammalian hearts Schenk (1924) and Katz and Long (1925) have obtained somewhat lower figures than these.

SUMMARY

The ability of the ventricular muscle of the turtle to develop tension is closely correlated with its content of lactic acid.

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A DYE-METHOD FOR DETERMINING THE BLOOD VOLUME IN MAN

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In a previous paper I have published a series of in-vitro experiments undertaken in order to test the reliability of the dye-methods for determining the blood volume as indicated by Keith, Rowntree and Geraghty (1915) and adopted by a number of observers in experiments on man as well as on animals. These experiments have shown that bloods from different species behave in a different manner; in some species a very grave error on the plasma determination is met with, caused by the fact that dye-substance is adsorbed to the red cells. This error, which is counteracted by dilution of the blood, is very great in ox-blood, it is less though still considerable in dogs' blood, and in the case of human blood our preliminary experiments showed no *systematic* error on the plasma determination. On the other hand, we found a certain variation in the results, indicating that some or other individual source of error might be present. We were inclined to believe that these uncertainties were due to the unavoidable errors caused by the manipulations required by the method; but later experiments have taught us that the source of errors in question must be sought in the red cells too. In order to complete our preliminary experiments with experiments on blood samples containing higher percentages of red cells we have undertaken a little series of experiments in vitro on quite small samples and have found that if dye-solution is added to the total blood the results spread as previously described thus proving the mutual independence between the error on the plasma determination and the number of the red cells, while on the other hand, if dye-solution was added to *plasma*, we were able to determine the plasma volume colorimetrically without any appreciable error, even if only very small samples were used. In spite of these discrepancies we have found it justifiable to endeavor to work out a reliable dye-method for clinical purposes; the following will make it clear why the routine hitherto adopted by clinicians cannot be accepted.

Two dye-substances have been tried: Vital red and Congo red, but only the first named suited the purpose; Congo red always caused a change in the shade of color which made colorimetry extremely unreliable, even if

control plasma were used, and whether the determination was carried out in daylight or in artificial light.

Starting from the supposition that the dye-substance is eliminated very slowly from the blood, as shown by previous observers, we directed our attention to certain points which seemed to us to be of special importance:

As the dye-solutions, when injected into the cutaneous veins of human subjects, for various and partly unknown reasons, sometimes cause dangerous or at least very disagreeable symptoms, the amount of dye-substance injected should be reduced to a minimum.

As the behavior of the dye-substance in the blood is altered by dilution, the blood samples must never be diluted.

As hemolysis renders the determinations fallacious, every plasma-portion must be examined spectroscopically before it is placed in the colorimeter.

As turbidity of the plasma, caused especially by lipemia, renders the reading of the colorimeter difficult or impossible, the determination of the blood volume must take place in the post-absorptive state of the subject.

The routine of the experiment proper was accordingly as follows:

Just before the beginning of the experiment the subject was told to walk up and down the floor, in the manner to be described below, for a few (4 to 5) minutes; then he was placed in a chair, and about 2.5 cc. of blood were drawn from his cubital median vein by means of a "Record"-syringe with a rather fine needle and containing an adequate amount of powdered oxalate. The blood sample was divided into two centrifuge glasses as described in a previous paper, stoppered by rubber stoppers, and placed in a centrifuge. Some 3 cc. (varying from 2.5 to 4 cc. according to the size of the subject) of a 1 per cent solution of vital red were injected into the same or the opposite cubital vein. About 5 minutes after the injection a second blood sample of about 2.5 cc. was drawn from the cubital vein opposite the place of injection in the manner described above. In the 4 to 5 minutes between the injection and the drawing of the second sample the subject was again walking up and down the floor.

After centrifuging for 1 hour at a speed of nearly 3000 revolutions per minute the volume of the red cells was read off; 1 cc. of the colored and uncolored plasma respectively was placed in the two closed cells of a Bürker colorimeter; then the two open vessels of the apparatus were filled with distilled water and a standard dye-solution respectively, the colorimeter was read off, and the result calculated. The following example will show the calculation:

Subject J. L. Injected 3 cc. of dye-solution. Hematocrit 45.5 per cent.
Standard: 0.2 per cent of the dye-solution. Colorimeter reading 9.9.
Plasma volume $3 \times 100/0.2 \times 10/9.9 = 1515$ cc.
Blood volume $1515/54.5 = 2.8$ liters.

We will submit the individual steps of the whole procedure to a more close examination.

Previous observers have varied the concentration of the dye-solution. Thus Keith, Rowntree and Geraghty (1915) used a 1.5 per cent solution, Griesbach (1921) a 1 per cent and Bennhold (1923) a 1 per cent or a 0.75 per cent solution. The solutions are sterilized by boiling. It is emphasized by Bennhold, working with Congo red, that the solution must be complete, if minor grains of the dye-substance are left undissolved, the injection will give rise to unpleasant symptoms to the subject, especially shivering. The volume injected is likewise subject to variation: 10 cc. of a 1 per cent solution (Griesbach, Bennhold), 15 cc. of a 0.75 per cent solution (Bennhold), or 3 mgm. per kgm. body-weight of a 1.5 per cent solution (Keith, Rowntree and Geraghty).

As mentioned above, we always injected about 3 cc. of a clear 1 per cent solution of vital red. At the beginning of the experimental series the dye-solution was sterilized by boiling for some 10 minutes. It happened, however, that the injection in two cases gave rise to severe symptoms; in the one the body temperature rose to about 40°C., and remained higher than normal for almost 48 hours. Hereafter a fresh dye-solution was made every third day and the solution was sterilized by boiling for some 5 minutes just before each injection. When this routine was followed, no toxic symptoms were observed. It seems to me that the most probable cause of the alarming symptoms is growth of bacteria in the dye-solution, and it is thus evident that the danger must be diminished, when the solution is used in a freshly prepared state and in a small quantity. Another reason to reduce the amount of dye-substance injected by previous observers is that injection of such quantities would render the color concentration too high for colorimetry, when undiluted blood is examined.

The blood samples are most conveniently drawn by means of a fine needle. In the beginning of the series we made use of a comparatively big needle in order to avoid clotting of the blood, if the puncture of the vein was first unsuccessful and had to be repeated. In a number of subjects, however, the puncture gives rise to a definite sensation of pain, and in such cases it is preferable to have a needle as thin as possible. Moreover, such a needle renders the puncture itself more easy to perform, especially if the cutaneous veins are small as is often the case in female subjects. The veins are made more easy to see or feel, if the blood stream is stopped for a short while, just before the puncture, by means of a tourniquet applied to the arm or leg. As soon as the puncture is performed, the compression is suspended.

The most essential point in the whole determination is, however, to secure a reliable mixing of the dye solution with the blood. So far as I am aware, previous observers have paid too little attention to the circula-

tion in the cutaneous veins; the reasoning usually met with is that within 4 or 5 minutes the normal circulation will suffice to secure a reliable mixing of the whole mass of blood, and this may be correct in the main; but regarding the cutaneous veins it is far from being true. If no special precautions are taken, the composition of the cutaneous venous blood may vary to a very considerable extent (cf. Barcroft and Nagahashi (1921)). In the first instance we found that the second blood sample, if drawn from the vein into which the dye solution had been injected, presented a relatively high concentration of the dye substance and accordingly gave strikingly low values for the blood volume. This being so, the drawing of the blood sample from the opposite arm must lead to too high values for the blood volume. In order to test this point further two blood samples were drawn nearly simultaneously from the cubital vein opposite the place of injection and from the great saphenous vein just proximal to the internal malleolus. We found:

Subject S. M. in ordinary sitting position.	*liters*
Blood volume calculated from cubital blood	3.7
Blood volume calculated from saphenous blood	8.0

This result shows conclusively that the routine adopted by previous observers is far from being reliable.

In order to secure a thorough mixing of the dye solution injected with the blood and, especially, to obtain a sufficient circulation in the cutaneous veins the subject was told to move in the time-interval between the injection and the drawing of the second blood sample. It is a well-known fact that the cutaneous veins of the arm, fore-arm and hand are emptied, when the arm is elevated to the vertical position, and filled, when the arm is hanging down; it has also been observed repeatedly that the strongly filled cutaneous veins of the dorsum of the foot are emptied almost instantaneously, when the subject is walking only a few steps. As soon as the injection had taken place we therefore told the subject to walk slowly up and down the floor and, at short intervals, to elevate his arms to the vertical position without any unnecessary muscular effort and, after a second or two, to drop them down again to the hanging position; care was taken that no hindrance to the cutaneous circulation was formed by the clothing, especially that of the arms and legs. Also protracted static action of the arm muscles or a sharp bend in the elbow-joint such as in folding the hands behind the neck, proved to be deleterious to the result and must, therefore, be avoided. To test again, if these precautions were necessary and sufficient, we made two experiments with one and the same subject the results of which are given below:

Subject P. E. Expt. I. 14/5. The subject during the whole experiment was sitting quietly in a chair with the legs resting on another chair.

	liters
Blood volume calculated from the cubital blood	4.7
Blood volume calculated from the saphenous blood	7.25

Expt. II. 16/5. The subject walked as described above for 5 minutes before drawing the second blood sample.

	liters
Blood volume calculated from the cubital blood	2.8
Blood volume calculated from the saphenous blood	2.8

It is not sufficient, however, to secure a homogeneous mixing of the dye-solution with the blood; we found repeatedly that the color proper of the plasma changed during the course of the experiment, especially when the subject had come to the laboratory after bicycling or fast walking, and, as differences in the shade of plasma color render the use of control plasma in the colorimeter illusory, it is essential to get rid of them; we therefore always took care to obtain a thorough mixing of the subject's blood by means of the movements described just before the beginning of the experiment.

Regarding the time necessary for complete mixing of the blood and dye-solution experiments have shown that 3 minutes are insufficient, while 5 are adequate; the upper limit of the interval between the injection and the drawing of the second blood sample cannot be determined with certainty; it is evidently, for reasons to be mentioned below, variable, as the elimination of dye-substance from the circulating blood takes place at an increasing rate, when the experiments are repeated on the same subject. It is essential, therefore, that the second blood sample is drawn about 5 or 5 to 6 minutes after the injection.

According to the above we may conclude that the precautions taken by us to secure a thorough mixing of dye-substance and blood are sufficient, and on the other hand, that if no such precautions are taken, the results obtained by any dye-method have no value at all.

Regarding the centrifuging it must be borne in mind that the glasses containing the blood must be stoppered; when blood at body temperature is centrifuged for an hour the volume, if the glasses are not stoppered, will decrease by evaporation and thus render the hematocrit reading fallacious. On the other hand, when no evaporation takes place, the reading of the volume of the red cells on the centrifuge glasses gives the same result as does the reading of an ordinary capillary hematocrit tube, when centrifuged to constant volume and translucency. The reading is reliable within 1 per cent, and as a rule no difference is found between the readings of the colored and the uncolored blood from the arm veins, but occasionally we have seen differences of 1 to 2 per cent in the cell volume of blood from nearly simultaneous samples from cubital and saphenous veins It cannot be decided whether the relative volume of the red cells is constant throughout the whole mass of blood, but the investigations at hand

seem to show that in the capillaries at least it may be varying; also in the larger veins real differences are occasionally found as indicated by the figures above. A difference of 2 per cent in two nearly simultaneous samples is undoubtedly beyond the limits of error; it cannot be denied, however, that slight variations in the blood volume may take place within the couple of minutes elapsing between the drawing of the two samples. At all events nothing compels us to assume that systematic errors are to be found, caused by variations in the cell volume, and the accidental errors, as will be shown below, are by no means grave enough as to restrict the value of the method to any appreciable extent. An error on the hematocrit reading of 2 per cent causes an error on the calculated blood volume of 3 to 4 per cent.

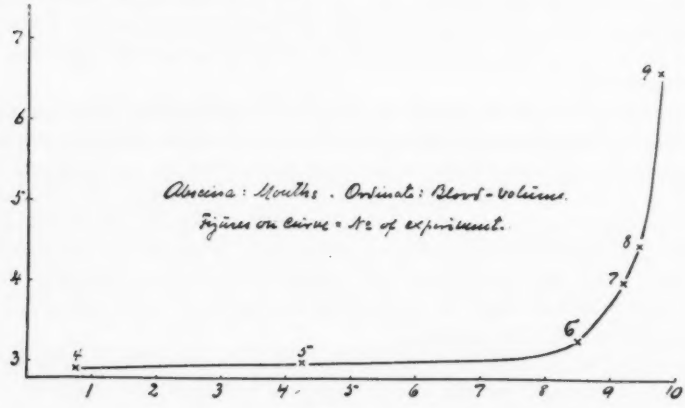

Fig. 1

For the experienced observer the error on the colorimeter reading amounts to 1 or 2 per cent, but this error may be reduced and a valuable control obtained, if the concentration of the standard solution is changed and the reading repeated. The error on the preparation of the standard solution may be taken as at most 1 per cent, and the same limit is valid also for the measuring off of the dye solution in the syringe. Errors on the preparation of the 1 per cent dye solution are negligible, when we always make use of a standard solution prepared from the dye solution injected. If, by misfortune, dye solution is injected into the perivascular tissue, we find on the following day a very intensive red coloring of the skin in the environment of the place of injection. When this is the case, the amount of dye solution really injected into the blood is too small, and the blood volume calculated is accordingly too high. If, therefore, the skin appears colored to any appreciable extent, the whole ex-

periment must be discarded. However, a perivascular injection of say 0.1 cc. of dye solution, which would suffice for coloring the whole arm, only involves an error of about 3 per cent, when the routine here described is followed.

It is impossible, however, from the above considerations to determine the uncertainty which appears in the practical use of the method. This uncertainty, caused partly by the above named sources of error, partly by the physiological variation of the function examined under the given experimental conditions, is shown in the table below containing double determinations on 11 healthy male subjects.

TABLE 1

SUBJECT*	BLOOD VOLUME IN LITERS	MEAN VALUES OF DOUBLE DETERMINATIONS			$W^{0.71}/B$
		In litres	In per cent of body weight	In per cent of body surface	
1	3.1–3.2	3.15	5.0	1.62	6.02
2	2.9–3.1	3.0	4.35	1.44	6.82
3	2.9–3.0	2.95	4.35	1.44	6.78
4	2.7–2.8	2.75	4.35	1.41	6.89
5	3.0–2.9	2.95	4.5	1.47	6.62
6	2.9–2.9	2.9	4.2	1.40	6.95
7	3.0–3.0	3.0	5.2	1.64	5.92
8	4.4–4.45	4.425	5.4	1.92	5.12
9	3.6–3.8	3.7	5.0	1.70	5.75
10	5.25–5.4	5.325	5.9	2.16	4.57
11	4.1–4.2	4.15	5.7	1.93	5.06

* These experiments will be mentioned in detail elsewhere by *Dr. C. C. Fleischer-Hansen* who has partaken in the investigation.

When for each pair of experiments we determine the deviation from the mean value, the average deviation is found to be 0.05 ± 0.01 liter, and if we calculate the blood volume in per cent of the body weight, we will find figures ranging from 4.2 to 5.9 on an average 4.9 ± 0.2 per cent, while the ratio (blood volume in liters)/(body surface in square-meters) is on an average 1.65 ± 0.08; Meeh's formula being used for the calculation of the surface. When the blood volume is known, we are able to calculate the constant K in the formula of Dreyer and Roy (1912), $B = W^{0.70-0.72}/K$; in this formula B means the blood volume, W the body weight and K an empirical constant which has been calculated by Dreyer and Roy for a number of species. If we calculate K from the experiments on man quoted above, we find K varying within the limits 6.95 to 4.57, thus indicating either that our results are very fallacious, or that the formula of Dreyer and Roy cannot be used with any advantage for determining the blood volume in man. The mutual agreement of the double determinations shows that serious accidental errors, due to the

experimental technique, are out of the question; but, on the other hand, it cannot be decided whether systematic errors are present or not. Now it is very difficult to imagine how we should obtain too low values for the blood volume; incomplete mixing of the dye solution with the blood might occasionally have this effect, but it cannot cause any systematic error. Certainly, a systematic error might arise, if a certain fraction of the total blood was withdrawn from the general circulation and thus escaped mixing with the dye solution, and according to the recent investigations of Barcroft and his co-workers, we must admit that this possibility may be realized in the spleen; but on the other hand, the error possibly introduced in this way is presumably only slight, the average weight of the spleen in man being only about 170 grams and, moreover, we must suppose that the exercise taken by the subject after the injection of the dye solution would rather tend to diminish the size of the spleen. Other sources of error tend to cause the dye concentration of the plasma to diminish and thus render the calculated blood volume too high. This is true if dye-substance is lost in some way or other through the walls of the vessels, if it is excreted through the kidneys or altered chemically by other circulating substances; or if it is adsorbed to the blood cells. Now previous investigators have shown that in the first minutes after the injection the amount of dye-substance excreted through the kidneys or lost through the walls of the vessels is exceedingly small, and I have in a previous paper made it probable that the dye-substance is not to any appreciable extent taken up by the red cells in human blood, but it cannot be denied that the white cells in the coloured blood appear of a more intense color than does the plasma. As, however, the leucocytes only amount to a fraction of 1 per cent of the plasma volume an error of this origin may undoubtedly be neglected.

Some light may perhaps be thrown on the question of systematic errors, if we compare our present results with those of previous observers. However, the number of comparable results is rather limited. The experiments on man undertaken according to the dye-methods cannot be discussed, because of the fact that no precautions have been taken to secure a reliable mixing of blood and dye-solution, and the same objection may perhaps be raised against the experiments on dogs by Whipple and his co-workers (1921); moreover in these experiments the hematocrit readings are without doubt too high, owing to the fact that the blood was centrifuged only for 25 minutes at a speed of 3000 revolutions per minute. The American observers found the relative cell-volume to be on an average 50 per cent, while we in 22 cases of dog's blood found a cell-volume of only 40 per cent. It seems to me very unlikely that this difference is real, owing to a different composition of the blood of American and Danish mongrels, and if it is due to incomplete centrifuging only, it is fatal to the

American results. It may be added further that, as I have shown in a previous paper, we have an error on the plasma determination in dog's blood increasing with increasing cell-volume and probably due to adsorption of the dye-substance to the red cells.

It is further a matter of fact that the results obtained according to the method of Welcker must be too high, because the results comprise also the extravascular hemoglobins, the amount of which is unknown but is usually estimated to some 10 per cent of the circulating hemoglobin. Certainly, an error of these dimensions would suffice to bring the results obtained by the Welcker method nearly on line with our results.

Regarding the CO-experiments of Haldane and Lorrain Smith (1900) it has been a matter of discussion, whether the carbon monoxide during the experiment diffuses from the vessels to the extravascular hemoglobin. Such a diffusion would render the results of the method too high. On the other hand, it has been doubted, whether the partial saturation of the hemoglobin with carbon monoxide has been the same throughout the whole mass of blood. If this has not been the case, serious errors may have arisen, but, on the other hand, it cannot be supposed that errors due to incomplete mixing would be systematic.

Haldane and Lorrain Smith have examined 14 healthy male subjects by means of the CO-method, and their results are thus directly comparable with ours.

	NUMBER OF SUBJECTS	NUMBER OF EXPERIMENT	BLOOD VOLUME IN PER CENT OF WEIGHT	LIMITS
H. and L-S.....	14	14	4.9	3.3-6.3
Present paper.....	11	22	4.9	4.2-5.9

Thus the two methods give exactly the same average value for the blood volume expressed in terms of the body weight. The range of variation is less in our experiments than in the series of the English authors, a difference which may be due to the rather complicated technique of the CO-method, but on the other hand can be due merely to chance. More extensive investigations will decide this question; but it seems to me that the fact that exactly the same values are arrived at by means of two fundamentally different methods furnish a very strong support to the results which cannot be far from truth. In the one case the amount of hemoglobin is determined by means of a diffusible gas introduced in the lungs; in the other case the amount of plasma (+ white corpuscles) is determined by means of a non-diffusible dye-substance introduced in the blood vessels. The sources of error are quite different in the two methods; when the effect of the errors on the results is equal, it is justifiable to conclude that it must be very small.

According to the above it is most probable that CO does not to any appreciable extent diffuse to the extravascular hemoglobin during the experiment; later experiments by Douglas and Boycott (1909) have shown, however, that the CO-method gives much higher figures for the blood volume, if the second blood sample is drawn 15 instead of 2 minutes after the inhalation of CO is finished, and these higher values have been accepted by Haldane (1922) as more closely agreeing with the result of Welcker. Now the method of Welcker as mentioned above *must* give too high values for the blood volume, owing to the extra-vascular hemoglobin, and there can be no doubt, therefore, that the better agreement between the CO-method and that of Welcker obtained by the authors named is due to diffusion of CO to the extravascular hemoglobin, and, the purpose being to determine the blood volume and not the total hemoglobin in the body, we must hold that the true figures for the blood volume are obtained by means of the dye-method as described above and by the CO-method as first used.

As the CO-method is much more complicated and laborious than is the dye-method, the choice of method would be easy if the dye-method did not suffer from a very strong limitation. Repeated determinations of the blood volume on the same subject have shown that the subject acquires a sort of "immunity" for the dye-substance. When this state has been reached, the dye vanishes from the blood at an increasing rate, and always so fast that a thorough mixing cannot be attained. In three subjects, on whom 6 experiments were made within 10 months, the "immunity" was evident in the seventh experiment; in the sixth experiment the blood volume was only slightly increased; but when the interval between the experiments is only some few days, "immunity" may be attained as early as the fourth experiment. This "immunity" is not due to the formation of antibodies in the blood,—it is possible to determine a blood volume in vitro in blood from an "immune" subject,—but the cause must be sought in certain body cells, perhaps in the liver and the spleen, and this is the reason why in "immune" subjects complete mixing of dye solution and blood cannot be attained. Once acquired the "immunity" increases at a very steep curve, as shown in figure 1; in this case it is manifest in the seventh experiment, but there is no doubt that it may be traced back to the first injection. A close examination of table 1 will show that in 8 out of 11 double determinations the second observation is higher than the first, in two cases the values are equal, and in one case only the first is the higher. The differences are so small, however, that we failed to appreciate them until the peculiar "immunity" was definitely recognized.

To test the specificity of this biological reaction it was attempted to inject another dye in a subject "immune" for vital red. The attempt was made with blue dye (T 1824) recommended by the American authors,

but the result was not encouraging; the figure for the blood volume fell on the same line as did those from the vital red experiments. It is most probable therefore, that we must accept this limitation for the dye methods and make use of other methods if we want to obtain experimental series comprising more than some five observations. In certain cases, however, the dye methods are alone suitable: in pregnancy we must assume that the conditions for the diffusion of gases from the maternal to the fetal blood are extremely favorable, and under these circumstances one cannot rely upon the CO-method, if we want to determine the blood volume of the mother, while vital red in all probability is unable to penetrate the endothelial cells lining the placental blood spaces.

SUMMARY

A dye-method for determining the blood volume in man is described in detail and discussed.

It is shown to be absolutely essential that the blood of the subject is completely mixed with the dye injected by a suitable routine of movements.

Double determinations on the same subject agree within 0.2 liter, on an average 0.05 ± 0.01 liter.

The total blood volume in 11 healthy male subjects is found to be on an average 4.9 per cent of the body weight with individual variations ranging from 4.2 to 5.9 per cent.

When dye-substances are injected repeatedly the subject after some few injections acquires the property of eliminating the dye from the circulation, thus rendering further application of the method illusory. The number of injections leading to "immunity" varies with the length of the intervals; at most 5–6 determinations may be performed in the course of a year. The "immunity" does not seem to be specific.

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NOTE UPON THE PRESENCE OF HEPARIN IN NORMAL AND HEMOPHILIC BLOOD OF MAN

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In a recent paper the author (1925) has given evidence to show that the delayed clotting or incoagulability of blood of a peptonized dog is caused by the presence of an excess of heparin in the blood. I have shown, moreover, that in normal dog's blood a detectable amount of heparin is present. The method used to demonstrate these points was briefly as follows: The blood received from an artery was oxalated and centrifugalized. In some cases the peptonized blood was centrifugalized without oxalating. The clear plasma was pipetted off and precipitated by the addition of an equal volume of acetone. The precipitate was collected by centrifugalization and was boiled for two hours in a large volume of 95 per cent methyl alcohol, using a reflux condenser. The solution was filtered, washed with hot methyl alcohol and the precipitate was dried and powdered. This material was then extracted for 15 to 30 minutes with a solution of 1 per cent sodium chloride, the extract was filtered and the filtrate was precipitated by an equal volume of acetone. This precipitate was collected by centrifugalizing and dried. It was then extracted with a little water, filtered and the filtrate evaporated to dryness over the water-bath. The residué thus obtained was used for testing for the presence of heparin.

More recently this method has been varied with advantage in the following way: The whole blood has been used in place of the centrifugalized plasma. In the case of human blood the procedure has been to insert a good-sized hypodermic needle into an arm vein and allow the blood to flow through an oiled tube directly into acetone. Usually 100 cc. of blood were taken from the vein and received into 100 cc. of acetone, the mixture being stirred gently during the flow. The precipitate was separated by centrifugalizing and was boiled for two hours with an excess of methyl alcohol. The solution was filtered, the precipitate was washed with hot methyl alcohol, dried and powdered. The dried material was then extracted for a few minutes with a boiling solution (150 cc.) of 1 per cent sodium chloride. The filtered extract was precipitated by acetone and treated as described above.

With the small amount of material thus obtained tests were made for the presence of heparin in human blood as follows. The entire material

was extracted with 2 cc. of a 1 per cent solution of sodium chloride. The extract was filtered and the filtrate was compared with an equal volume of 1 per cent sodium chloride solution in regard to its property of delaying the coagulation of freshly drawn blood and of causing an increase in antithrombin when added to oxalated plasma.

Anticoagulant action. The anticoagulant action was tested by mixing 0.5 cc. or 1 cc. of the filtrate with 1 cc. of normal human blood taken from an arm vein by means of a syringe:

Experiment V. Control: 0.5 cc. salt solution plus 1 cc. blood = clot in 7 to 8 minutes.
Extract: 0.5 cc. extract plus 1 cc. blood = clot, imperfect, in 40 to 50 minutes.
Experiment VI. Control: 1 cc. salt solution plus 1 cc. blood = clot in 12 minutes.
Extract: 1 cc. extract plus 1 cc. blood = clot in 50 minutes.
Experiment VII. Control: 1 cc. salt solution plus 1 cc. blood = clot in 10 minutes.
Extract: 1 cc. extract plus 1 cc. blood = clot in 48 minutes.

Antithrombin effect. It is one of the characteristics of heparin that when added to serum or plasma it causes an increase in the content of antithrombin as tested upon definite mixtures of fibrinogen and thrombin. In these experiments oxalated cat's plasma was used after previously heating to 56°C. to destroy the fibrinogen. The thrombin was made from a dried specimen prepared by my method and now some 12 years old. Ten milligrams of the thrombin were dissolved in 15 cc. of 1 per cent solution of sodium chloride. The fibrinogen solutions were made from oxalated cat's plasma by the ammonium sulphate method. They did not clot spontaneously nor upon the addition of the calcium chloride and were, therefore, free from thrombin and prothrombin.

When the thrombin solution was added to the fibrinogen solution clotting occurred in a few minutes. The time varied in different specimens of fibrinogen, but was always short, for example:

Thrombin 4 drops + Fibrinogen 8 drops = clot in 2 minutes
Thrombin 3 drops + Fibrinogen 8 drops = clot in 2 to 3 minutes
Thrombin 2 drops + Fibrinogen 8 drops = clot in 4 to 5 minutes
Thrombin 1 drop + Fibrinogen 8 drops = clot in 8 to 9 minutes

In the test for antithrombin in the heated oxalated plasma two solutions were prepared—one, a control, consisted of equal parts of the heated oxalated plasma and a 1 per cent solution of sodium chloride, the other, of equal parts of the heated oxalated plasma and the extract prepared from the normal human blood as described above.

Experiment VII. Antithrombin in the control mixture:
Thrombin 1 drop + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot in 5 hours
Thrombin 2 drops + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot in 25 minutes
Thrombin 3 drops + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot in 15 minutes

Thrombin 4 drops + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot in 10 minutes

Antithrombin in the blood extract mixtures:

Thrombin 1 drop + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot between 5 and 24 hours

Thrombin 2 drops + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot between 110 and 130 minutes

Thrombin 3 drops + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot in 25 minutes

Thrombin 4 drops + solution 1 drop, incubation 15 minutes, fibrinogen 8 drops = clot in 20 minutes

The antithrombin content of the heated oxalated plasma was, therefore, increased by the addition of the extract prepared from normal blood. That this extract did not itself contain any antithrombin was demonstrated by a control experiment, as follows:

Thrombin 1 drop + blood extract 1 drop, incubation of 15 minutes + fibrinogen 8 drops = clot in 7 to 8 minutes

Thrombin 2 drops + blood extract 1 drop, incubation of 15 minutes + fibrinogen 8 drops = clot in 3 to 4 minutes

Thrombin 3 drops + blood extract 1 drop, incubation of 15 minutes + fibrinogen 8 drops = clot in 3 to 4 minutes

Control:

Thrombin 1 drop + saline 1 drop, incubation of 15 minutes + fibrinogen 8 drops = clot in 6 to 7 minutes

Thrombin 2 drops + saline 1 drop, incubation of 15 minutes + fibrinogen 8 drops = clot in 3 to 4 minutes

Thrombin 3 drops + saline 1 drop, incubation of 15 minutes + fibrinogen 8 drops = clot in 3 to 4 minutes

It seems certain from these results that normal human blood contains an anticoagulant different from antithrombin. The method by which it was obtained and its power to increase the antithrombin content of serum or plasma serve to identify this anticoagulant with heparin.

Hemophilic blood. The fact that the heparin in the incoagulable blood of the peptonized dog, as tested by the above methods, is greatly increased suggested the possibility that the delayed clotting of hemophilic blood may be due to the same cause. Weil (1906) was the first to suggest that the delayed clotting of hemophilic blood is due to the presence of an excess of some anticoagulant. In two out of three cases he found that the serum of hemophilic blood delayed the clotting of normal blood. Other authors have not been able to confirm this result. Addis (1911) obtained entirely negative results. The addition of oxalated hemophilic plasma to oxalated normal plasma did not delay the clotting of the latter when recalcified. Howell (1914) examined the blood of hemophilics for the presence of antithrombin, by his method, as compared with normal bloods. While, in general, an excess was found the difference was slight

and in some cases was lacking. A similar result was obtained by Minot and Lee (1916).

As further proof of the absence of an excess of anticoagulant, Feissly (1923) states that thrombin added to hemophilic plasma causes it to clot as readily as it does normal citrated plasma. This argument applies, however, only to an anticoagulant of the nature of antithrombin, and not to heparin whose effect is not exerted upon thrombin but upon the antecedent substance, the prothrombin. In another paper Feissly (1925a) states that when prothrombin (proserozyme) is prepared from hemophilic plasma by the Bordet method of adsorption upon tricalcium phosphate, this precipitate, when redissolved in saline, possesses the property of preventing the coagulation of a recalcified citrated plasma, and in a general discussion of the subject he considers the possibility of the existence of some stabilizing or inhibitory factor in hemophilic blood (1925b).

Davidson and McQuarrie (1925) state that a suspension of platelets prepared from hemophilic blood when added to normal oxalated plasma causes a marked prolongation of the coagulation time upon recalcification, whereas a suspension of normal platelets has no such effect. This observation, however, is contradicted by the similar experiments of Feissly and Fried (1924). They found that suspensions of platelets from hemophilic plasmas had the same effect as similar suspensions from normal plasmas in hastening the coagulation of a recalcified oxalated plasma.

Minot and Lee (1916) obtained the same result when the optimum amount of calcium was used for recalcification.

There is obviously some disagreement among workers in regard to the existence in hemophilic blood of an anticoagulant material in excess of that present in normal blood. So far as antithrombin is concerned, the direct evidence quoted above indicates that it is not present in amounts distinctly above normal; but since I have shown that normal blood contains another anticoagulant, heparin, which is an antiprothrombin, it seemed desirable to study hemophilic blood with reference to its content in this latter substance, using the methods described above. Two young men, J. Y. and F. Y., belonging to the hemophilic family that I studied in 1914, were used in these experiments. These individuals had been kept under observation during the intervening period. From time to time they have had trouble from spontaneous hemorrhages in the joints or kidneys and have been treated by transfusion. One of them, J. Y., at the time of these experiments had been undergoing treatment for six months consisting of periodic injections intravenously of calcium chloride. While the result of this treatment seemed to be beneficial and the coagulation time of his blood was reduced somewhat, his general condition was not entirely satisfactory. When examined in 1913 the coagulation time of J. Y.'s blood was from 3 to 4 hours, examined again in 1924 it was 2 hours. After the above treatment had been continued for six months the coagulation time

was from 1½ to 2 hours. The coagulation time of the blood of F. Y. taken in February and March, 1924, was from 1 hour, 15 minutes to 1 hour, 40 minutes. At the time that these experiments were made, May and June, 1925, the coagulation time was much longer, approximately 4 hours. During the following summer this case was taken into the hospital in consequence of serious hemorrhages from the kidney and the knee-joint and was given several transfusions. It should be stated that the coagulation time was determined in all cases by taking a specimen from the arm vein with a syringe and emptying 2 cc. into a clean tube, having a diameter of 15 mm.

Blood (100 cc.) was taken from the arm vein of these two hemophilics, by means of a syringe or through a needle and tube. In some cases it was oxalated and centrifugalized to get the plasma. In other cases it was run directly into an equal volume of acetone. The acetone precipitate was treated as in the case of the normal blood to obtain the heparin, and the final material was examined for its anticoagulant action and its property of increasing the antithrombin content of heated plasma in comparison with an equal specimen of normal blood treated by the same method.

The results were not consistent. In some cases the hemophilic blood contained a greater amount of the heparin than the normal blood judged by the two tests described, while in other cases it contained practically the same amount or even less. The hemophilic blood seemed to vary somewhat in its content of heparin and as between the two hemophilic cases examined the blood of F. Y. contained uniformly more than that of J. Y. Without giving the full details of the experiments, the general results were as follows:

Anticoagulant action. The anticoagulant action was tested by mixing 1 cc. of the extract with 1 cc. of freshly drawn blood. In the control 1 cc. of a 1 per cent solution of sodium chloride was used in place of the extract.

	CONTROL	HEPARIN EXTRACT FROM NORMAL HUMAN BLOOD	HEPARIN EXTRACT FROM HEMOPHILIC BLOOD
	minutes	*minutes*	*minutes*
Experiment I. Using cat's blood for coagulation	28	45	50 (J. Y.)
Experiment II. Using cat's blood for coagulation	17	40	100 (J. Y.)
Experiment III. Using human blood for coagulation	27	27	45 (J. Y.)
Experiment IV. Using human blood for coagulation	10	23	28 (J. Y.) 35 (F. Y.)
Experiment VI. Using human blood for coagulation	12	50	40 (F. Y.)
Experiment VII. Using human blood for coagulation	10	48	28 (J. Y.) 80 (F. Y.)

Antithrombin action. Experiment I. Control:

Thrombin 1 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 20 minutes
Thrombin 2 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 15 minutes
Thrombin 3 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 15 minutes
Thrombin 4 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 10 minutes

Extract of normal blood:

Thrombin 1 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 25 minutes
Thrombin 2 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 15 minutes
Thrombin 3 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 15 minutes
Thrombin 4 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 10 minutes

Extract of hemophilic blood:

Thrombin 1 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 25 minutes
Thrombin 2 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 15 minutes
Thrombin 3 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 15 minutes
Thrombin 4 + extract 1—incubation 15 minutes + fibrinogen 8 = clot in 10 minutes

Experiment 2. Following the same procedure the clotting times were:

Control:

Thrombin 1 drop = clot in 50 minutes
Thrombin 2 drops = clot in 20 minutes

Extract of normal blood:

Thrombin 1 drop = clot in 60 minutes
Thrombin 2 drops = clot in 20 minutes

Extract of hemophilic blood:

Thrombin 1 drop = clot in 75 minutes
Thrombin 2 drops = clot in 25 minutes

Experiment IV. Control:

Thrombin 1 drop = clot in 8 to 9 hours
Thrombin 2 drops = clot in 120 minutes
Thrombin 3 drops = clot in 60 minutes
Thrombin 4 drops = clot in 25 minutes

Extract of normal blood:

Thrombin 1 drop = clot in 8 to 9 hours
Thrombin 2 drops = clot in 120 minutes
Thrombin 3 drops = clot in 60 minutes
Thrombin 4 drops = clot in 25 minutes

Extract of hemophilic blood:

Thrombin 1 drop = clot (beginning) after 14 hours
Thrombin 2 drops = clot in 165 to 190 minutes
Thrombin 3 drops = clot in 95 minutes
Thrombin 4 drops = clot in 55 minutes

Experiment VI. Control:
Thrombin 1 drop = clot after 18 hours
Thrombin 2 drops = clot after 18 hours
Thrombin 3 drops = clot after 18 hours
Thrombin 4 drops = clot after 105 minutes
Extract of normal blood:
Thrombin 1 drop = clot after 18 hours
Thrombin 2 drops = clot after 18 hours
Thrombin 3 drops = clot after 18 hours
Thrombin 4 drops = clot in 160 minutes
Extract of hemophilic blood:
Thrombin 1 drop = clot after 18 hours
Thrombin 2 drops = clot after 18 hours
Thrombin 3 drops = clot in 160 minutes
Thrombin 4 drops = clot in 55 minutes
Experiment VII. Control:
Thrombin 1 drop = clot between 5 and 7 hours
Thrombin 2 drops = clot in 25 minutes
Thrombin 3 drops = clot in 15 minutes
Thrombin 4 drops = clot in 10 minutes
Extract of normal blood:
Thrombin 1 drop = clot between 5 and 24 hours
Thrombin 2 drops = clot in 110 to 130 minutes
Thrombin 3 drops = clot in 25 minutes
Thrombin 4 drops = clot in 20 minutes
Extract of hemophilic blood:
Thrombin 1 drop = no clot after 24 hours
Thrombin 2 drops = clot between 5 and 24 hours
Thrombin 3 drops = clot in 40 minutes
Thrombin 4 drops = clot in 20 minutes

CONCLUSION

The results from both of these tests show that the hemophilic blood like the normal blood contains heparin in amounts that vary within certain limits, but there is no clear indication of a greater quantity of heparin in the hemophilic blood. While in some cases the heparin was greater than in the control of normal blood, the difference was not decisive, and in other cases there was no difference or the relation was reversed. The method used to extract the heparin was not quantitative, and might have been subject to unknown variables that confused the results. Still when the two specimens were put through exactly the same process, any definite excess of heparin in the hemophilic blood, if present, ought to have been made as clearly evident as in the case of the blood of the peptonized dog. The results, therefore, indicate that the hemophilic blood contains no excess of heparin beyond the normal limits, and the delayed coagulation can not be explained on this ground.

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CHANGES IN BODY TEMPERATURE AND METABOLISM ACCOMPANYING EXPERIMENTAL MARKED DIURESIS

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Continuous records of temperature were made in dogs subjected to marked diuresis. Previously we had shown that the administration of solutions of sugar (50 per cent) caused a loss of body water amounting to from 7 to 10 per cent of the body weight, and a maximal rise in body temperature of 3.4°C. The dogs recovered if water approximating that lost was administered within a few hours of the production of the diuresis (Keith, 1924). The temperature was determined at intervals of from fifteen to thirty minutes by an accurate clinical thermometer. In the present series of experiments continuous records of temperature were made over periods of from four to seven and one-half hours, and at the same time detailed studies of metabolism were carried out. Our object was to correlate changes in body temperature with the degree of water loss, with the quantitative changes of certain metabolites in the blood and the amount excreted in the urine.

EXPERIMENTAL PROCEDURE. The experiments were made on eight healthy female dogs whose urine was free from albumin. After certain disastrous results with dogs harboring intestinal worms (for example, dog H415, table 1), a vermifuge was given as a routine for two or three days previous to the experiment, until the stools were free from worms. No food was given on the day of the experiment or during the previous twenty-four hours. As much as 300 cc. of water was allowed during the twenty-four hours before the experiment. Continuous injections of a 50 per cent solution (100 gm. sugar in 200 cc. solution) of either saccharose or glucose were given intravenously, the amount injected being 7 to 9 grams for each kilogram of body weight each hour, for two hours. The best quality of commercial saccharose (highest grade of white granulated cane sugar) and the glucose issued by the Bureau of Standards, Washington, were used. Inorganic analysis of the saccharose and glucose failed to show any chlorid, potassium or calcium. Sodium was present in amounts of from 10 to 22 mgm. per cent. The weighed amounts of sugar were dissolved in

[1] In two experiments (dog G607, and H403) strict aseptic technic was not carried out, nor was the rubber tubing detoxicated.

TABLE 1

Water loss and temperature changes

DOG	DATE, 1924	WEIGHT, KGM.	SUGAR INJECTED EACH HOUR, GM. FOR EACH KGM.		RECTAL TEMPERATURE				PERIOD OF WATER LOSS			PERIOD OF RESTORATION		UNTOWARD SYMPTOMS	OUTCOME	NECROPSY FINDINGS
					Variation control period		Variation water loss period									
			Saccharose	Glucose	Degrees Fahrenheit	Degrees Centigrade	Degrees Fahrenheit	Degrees Centigrade	Duration, hours	Water loss for each kgm., cc.	Weight loss, kgm.	Water ingested, cc.	Water gained, kgm.			
H583	11–17	7.9	7				2.6	1.4	5	88	0.92	855	0.54	None	Satisfactory	
H580	11–24	7.9		8.0	0	0	2.0	1.1	5	69	0.69	750	0.43	None	Satisfactory	
H706	12– 3	5.25		8.5	0.6	0.3	2.2	1.2	4	47	0.36	450*	0.23	None	Satisfactory	
H714	12–10	6.63	8		1.3	0.7	3.8	2.1	4	99	0.81	605*	0.43	None	Satisfactory	
H415	11– 5	7.15		9.0	1.8	1.0	2.1	1.2	6	82	0.68	265		None	Death in nine hours	Numerous round-worms, mild enteritis
H581	11–11	10.0	8		1.0	0.5	3.8	2.1	5	78.5	1.03			None	Death in five hours	One round-worm, mild gastro-enteritis
G607	10– 7	11.5		9.0	0.9	0.5	12.4	6.9	1	95	1.17			Convulsions	Death in one hour	Three tapeworms, mild enteritis
H403	10–28	8.4	8		0.5	0.3	4.5	2.5	4.75	70	0.4			Convulsions	Death in four and three-fourths hours	Mild gastro-enteritis

* Distilled water.

sterile, triple-distilled water, in a sterile flask at 37° to 60°C. The flask containing the solution was then placed in a water-bath at 70°C. for ten minutes. The burette, rubber tubing, needle and syringe of the pump were dry-sterilized, so that when the injection was given continuously by means of the Woodyatt automatic pump, strict aseptic precautions were employed.[1] The rubber tubing was detoxicated by boiling in normal sodium hydroxid solution as recommended by Stokes and Busman (1920) and Busman (1920). The urine was collected accurately with a retention catheter, so that the water lost by diuresis was measured quantitatively. The body temperature was determined by placing the metal bulb of a Tagliabue continuously-registering thermometer[2] in the rectum, approximately 6 cm. above the anal sphincter. This remained in place throughout the experiment, although occasionally the passage of stool caused a temporary fluctuation in the tracing. The body temperature was determined for a control period of from two to four hours on a day previous to the diuretic experiment. Under the foregoing conditions there was marked water loss shortly after the injection was finished, but in order that the circulation might reach a state of equilibrium, the animal was left on the table and observed closely for the next three to five hours. At this time the loss of water and decrease in body weight were determined. The animal was then placed in a metabolism cage and permitted to drink measured quantities of tap or distilled water during the next twelve to eighteen hours. The total amount of water given closely approximated that lost by diuresis. Blood was drawn for analysis before the injection began, at the end of the two-hour injection period, at the end of the diuretic period, and on the following day. Urine was collected during the previous twenty-four hours, the injection period, and the diuretic and restoration periods. The bladder was always emptied by catheter at the end of each period.

[2] The Tagliabue thermometer used in these experiments was kindly lent by Dr. H. L. Dunn of the Department of Biometry and Vital Statistics of Johns Hopkins University who found that this particular instrument had an accuracy for the entire range of the temperature scale within 0.2°F.

Fig. 1. Continuous temperature charts, dog H580 (tables 1 and 2). Left chart, control. November 23, 1924; temperature registered continuously for two hours and forty minutes. Right chart, diuresis experiment, November 24, 1924; temperature registered continuously for seven hours and fifteen minutes.

Fig. 2. Continuous temperature charts, dog H714 (tables 1 and 2). Left chart, control, December 6, 1924; temperature registered continuously for three hours and fifty minutes. Right chart, diuresis experiment, December 10, 1924; temperature registered continuously for six hours and fifteen minutes.

Fig. 3. Continuous temperature charts, dog G607 (tables 1 and 2). Right chart, control, October 3, 1924; temperature registered continuously for three hours. Left chart, diuresis experiment, October 7, 1924; temperature registered continuously for four hours and twenty-five minutes. Note hyperpyrexia.

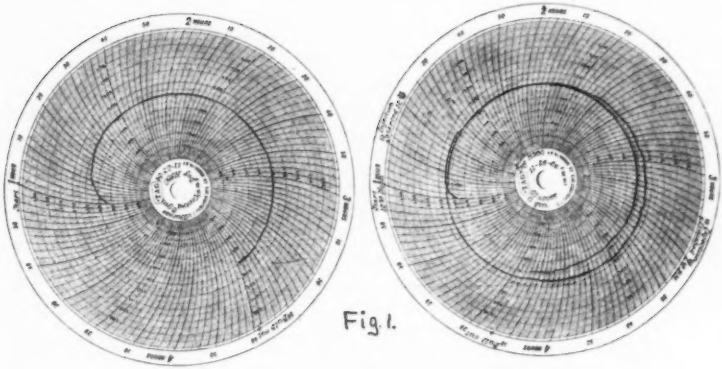

Fig. 1.

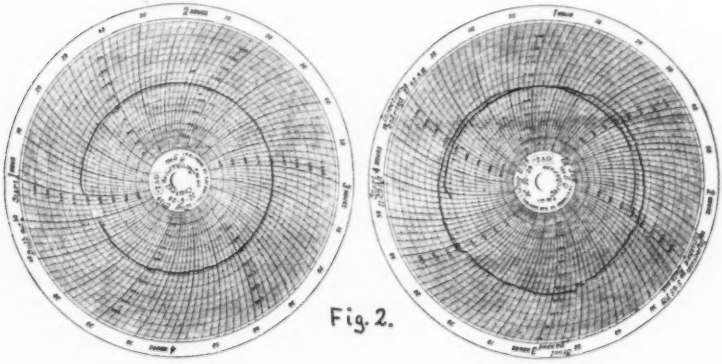

Fig. 2.

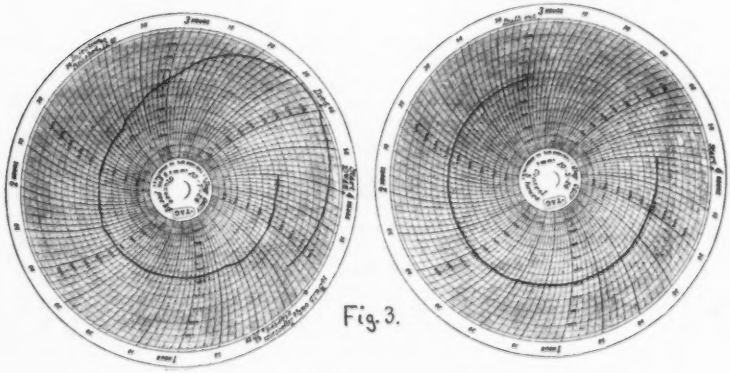

Fig. 3.

TABLE 2

Comparison of blood and urine findings

DOG	DATE, 1924	INTAKE OF WATER, 24 HOURS, CONTROL CC.	PERIOD*	BLOOD								URINE													
				Whole blood			Plasma	Serum							Urea nitrogen		Ammonia nitrogen		Chlorin		Sodium		Potassium		Phosphorus	
				Hemoglobin, gm., per cent	Glucose, mgm., per cent	Urea nitrogen, mgm., per cent	Carbon dioxid combining power, volumes per cent	Chlorin, mgm., per cent	Sodium, mgm., per cent	Potassium, mgm., per cent	Phosphorus, mgm., per cent	Volume, cc.	pH	Glucose, gm.	Gm., per cent	Total, gm.	Gm., per cent	Total, gm.	Gm., per cent	Total, gm.	Gm., per cent	Total, gm.	Gm., per cent	Total, gm.	Gm., per cent	Total, gm.
H583	11–17	300	1	15.9		12	45	371	335	18.4	3.9	85	6.4		1.87	1.59	0.20	0.17	0.84	0.72	0.48	0.40	0.25	0.21	0.18	0.16
			2	14.2		10						600	7.0		0.18	1 06	0.009	0.06	0.16	1.00	0.09	0.52	0.23	0.14		0.004
			3	17.7		16	50	421	420	18.1	6.0	330	7.0		0.12	0.40	0.00		0.09	0.30	0.04	0.14	0.05	0.16	0.006	0.023
			4	15.6		15	49	340	352	18.7	4.9	†				1.99	0.25									
H580	11–24	280	1	15.8	90	15	40	431	300	18.8	2.7	2‡	6.4	0.0					0.12							
			2	16.8	397	10						735	7.2	24.0	0.06	0.41			0.12	1.00	0.06	0.42	0.007	0.05		0.002
			3	21.6	82	18	46	459	322		7.4	65	7.2		1.86	1.21			0.06	0.04	0.02	0.016	0.007	0.005		Trace
			4	15.9	90	16	42	367	334		4.7	†				1.10										
H706	12– 3	200	1	16.7	84	16	52	423	343		4.7	130	5.8	0.0	2.98	3.87	0.23	0.3	0.60	0.80	0.20	0.26	0.100	0.130	0.100	0.110
			2	17.5	397	12						350	6.2	14.0	0.25	0.86	0.05	0.18	0.12	0.48	0.04	0.16	0.014	0.050	0.007	0.020
			3	20.0	108	18	58	445	367		6.5	65	6.0	0.8	0.09	0.06	0.0		0.08	0.05	0.03	0.02	0.006	0.004	0.013	0.008
			4	14.2	92	14	56	387	330		4.9	85	6.0	0.0	2.54	2.16	0.11	0.09	0.06	0.05	0.05	0.04	0.145	0.123	0.050	0.040

H714	12–10	200	1	15.7	108			416	397	21.6	5.1	100	5.6	0.0					0 29	0 29	0.09	0.09	0.110	0.110	0.110	0.110
			2	17.8	115							700	7.2	Trace					0.20	1.44	0.11	0.81	0.008	0.060	0.001	0.010
			3	24.4	105			405				160	7.0	Faint trace					0.04	0.06	0.03	0.046	0.004	0.006	0.001	0.002
			4	17.4	85			345	351			55	5.4	0.0					0.05	0.02	0.03	0.016	0.090	0.005	0.050	0.027
H415	11– 5	300	1	16.1	96	12	40	406	359	23.1	2.7	25‡	5.4	0.0	2.32		0.16		0.78		0.21		0.230			
			2	24.7	405	15	26	387			1.1	800	7.2	28.0	0.25	2.02			0.10	0.82	0.11	0.84	0.010	0.090		0.002
			3		68	10																				
H581	11–11	300	1	17.7		12	40	463	377	19.4	5.1	135	5.2		1.90	2.86	0.17	0.23	0.10	0.13	0.04	0.06	0.180	0.260	0.140	0.200
			2	16.1		14						850	7.4		0.19	1.63	0.01	0.11	0.18	1.60	0.09	0.76	0.020	0.160		0.003
			3	23.3			72	421	380	16.5	6.2	255	7.4		0.07	0.17	0.04	0.10	0.04	0.09	0.03	0.07	0.008	0.020		Trace
G607	10– 7	0	1	17.3	87	7	47	396	369	16.7		190	6.5	0.0	2.88	5.47	0.07	0.13	0.29	0.54			0.480	0.910		
			2	23.6	860	8	26					1,480	6.8	50.0	0.34	5.03	0.03	0.45	0.32	4.80			0.008	0.120		
			3	25.2	740	8	38	365	386			30	6.5	1.4	0.35	0.11	0.04	0.01	0.11	0.04			0.005	0.001		
H403	10–28	300	1	17.9	84	7	60	383	387	18.7		80	6.4	0.0	2.78	2.22	0.03	0.024	0.07	0.06	0.14	0.13	0.220	0.180		
			2	17.9	87	7	56	350	357	17.9		740	7.3	0.8			0.03	0.22	0.12	0.90	0.034	0.25				
			3									110	7.3	0.2					0.05	0.05	0.013	0.014	0.090	0.100		

* Blood specimens at end of period; urine collections total during period: (1) control period, (2) injection, (3) water loss, (4) restoration.

† Fourteen and eleven hour excretions of urine collected in two specimens, analyses carried out separately on each (see table 3).

‡ Incomplete specimens of urine.

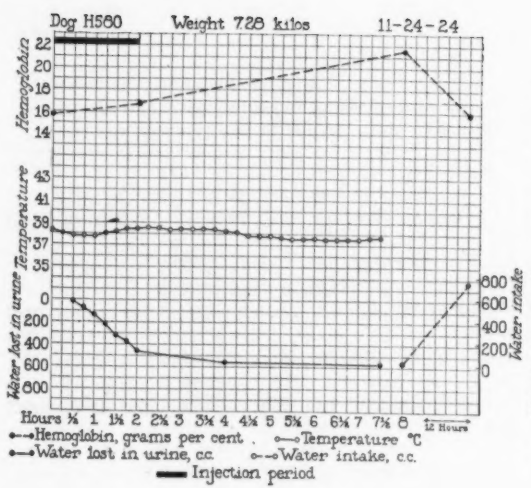

Fig. 4

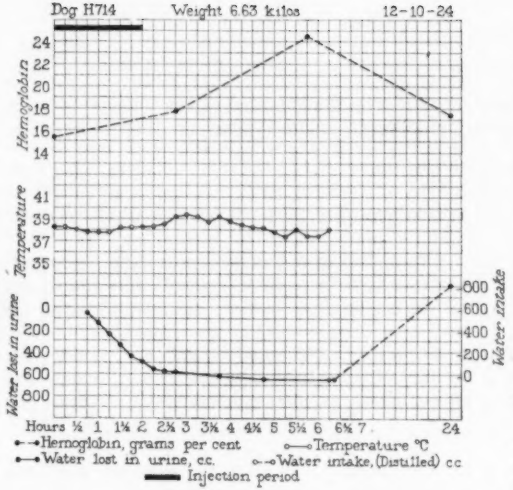

Fig. 5

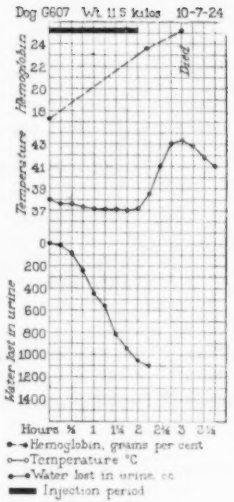

Fig. 6

Fig. 4. Dog H580. Relation between blood hemoglobin and body temperature during water loss and after restoration of water lost

Fig. 5. Dog H714. Relation between blood hemoglobin and body temperature during water loss and after restoration of water lost

Fig. 6. Dog G607. Relation between blood hemoglobin and body temperature during water loss. Note hyperpyrexia

EXPERIMENTS IN WHICH ANIMALS RECOVERED. Typical and satisfactory experiments were carried out in four dogs (H580, H583, H706, H714). No untoward symptoms developed during the experiments, and at the completion of the restoration period the animals appeared normal. They lost from 4.7 to 9.9 per cent of their weight through the urine, and had it restored, within twenty-four hours. During the control temperature period of two and one-half to four hours, the rectal temperature varied from 0 to 0.7°C. (Figs. 1 and 2 and table 1.) During the injection of the 50 per cent solution of sugar there was usually a slight drop in the temperature, but within the next half-hour a rise of 1.1° to 2.1°C. occurred, which persisted until the latter part of the experiment when a second drop occurred (figs. 1 and 2). This maximal rise in temperature was lower than that of 3.4°C. observed in our previous experiments (Keith, 1924). Thus water to the amount of 4.7 to 9.9 per cent of the animal's weight was removed with the slight change in temperature of 1 to 2°. These results agree closely with and confirm those obtained in previous experiments, when the temperature readings were only taken at stated intervals o fifteen to thirty minutes. Evidence of a decrease in the volume of the circulating blood was shown by a rise in hemoglobin concentration of from 111 to 155 per cent of that in the control blood. After restoration of the water the hemoglobin values fell and closely approximated the initial concentration, indicating restoration of the volume of circulating blood (table 2). The relation of hemoglobin concentration to body temperature, and to water lost and subsequently added, is shown in figures 4 and 5. We have shown previously the close relationship between measured blood volume and hemoglobin changes (Keith, 1924).

Studies on the chemistry of the blood (table 2) (4). In three of these experiments the carbon dioxid capacity of the plasma was determined No distinct change was observed. In dog H583, after a loss of water of 83 cc. for each kilogram of body weight the carbon dioxid capacity of the plasma was 50 per cent by volume. The blood sugar rose to 0.4 per cent after the administration of glucose, but fell to normal during the diuretic period. With saccharose there was no change in blood sugar as determined by the Folin and Wu method. The blood urea content followed a typical curve in three experiments; there was a fall at the height of diuresis, with a rise to above the control level with marked loss of water and a final approach to the initial value with restoration of water. In three experiments the chlorid content of plasma showed a rise of 5 to 13 per cent with marked diuresis. In the fourth experiment there was a slight decrease. By the administration of water to all four animals in an amount equal to that lost, there was a fall of from 13 to 20 per cent in plasma chlorid. Thus there was evidence in the plasma of a dilution of the chlorid to a level distinctly below that at the beginning of the experiment. The sodium

content of the plasma increased from 335 to 420 mgm. per cent in dog H583 with diuresis, but there was only a slight increase in dog H580 and dog H706. The actual content on the following day varied little from that of the initial control. In dog H583 there was no change in the amount of potassium in the serum throughout the experiment. The phosphorus content of the plasma at the time of most marked diuresis was uniformly increased over that of the control.

Studies of the chemical changes in the urine (5). The changes in the hydrogen-ion concentration of the urine were always the same; there was a decrease with excessive diuresis, and a return to the initial value when the water lost was restored to the organism. The glucose excreted amounted to from 16 to 19 per cent of the amount injected, and no reducing substance could be found in the urine on the following day. After an injection of saccharose only a small amount of a reducing substance was demonstrable in the urine. In two experiments (dogs H583 and H706) the urea in the urine was estimated on the day before and on the day of the experiment. In both instances the concentration was markedly reduced at the time of the diuresis but the total twenty-four-hour output approximated or was greater than that of the control day. With the ingestion of water there was rapid restoration of urea concentration in the urine to normal; this is worthy of note. The excretion of ammonia nitrogen was decreased during diuresis and the concentration became extremely low. In dog H583 (table 3) the concentration the morning after the experiment was equal to that of the control period and the restoration was similar to that of urea.

The total output of chlorid in the urine was usually increased and the concentration decreased. However, in the experiment on dog H706, both concentration and total excretion were decreased. In three experiments the total amount of sodium remained approximately the same with marked decrease in concentration during diuresis. In dog H714 there was a distinct increase both in concentration and total amount of sodium. The potassium excreted was more variable in amount than chlorid or sodium, but as a rule it was decreased. The variability in the excretion of chlorid, sodium and potassium under the conditions of these experiments is probably due, in part, to the variable amount of the inorganic ions present in the body at the time of diuresis. The characteristic feature of the phosphate excretion was that it practically disappeared from the urine during diuresis. Detailed urinary analyses in dog H583 are given in table 3. The sharp contrast between the rapid restoration of the urea and ammonia concentration, and the continued minimal excretion of the inorganic ions, chlorid, sodium, potassium and phosphorus, may be seen at a glance. Thus the restoration of water brought about normal excretion of urea and ammonia, but the marked decrease in these inorganic ions persisted. A similar decrease of these ions in the urine is characteristic of dog and man,

TABLE 3

Detailed findings in urine (dog H583)

DATE	TIME	VOLUME, CC.	pH	UREA NITROGEN		AMMONIA NITROGEN		CHLORIN		SODIUM		POTASSIUM		PHOSPHORUS	
				Grams, per cent	Total, gm.	Grams, per cent	Total, gm.	Grams, per cent	Total, gm.	Grams, per cent	Total, gm.	Grams, per cent	Total, gm.	Grams, per cent	Total, per cent
11–16–24 to 11–17–24	10:00 a.m. to 10:00 a.m. 24 hours	85	6.4	1.87	1.59	0.20	0.17	1.4	1.2	0.48	0.40	0.25	0.21	0.18	0.16
	10:52 to 12:52. Diuresis	600	7.0	0.18	1.06	0 009	0.06	0.3	1.6	0.09	0.52	0.02	0.14	0.001	0.006
	12:52 to 5:52.	330	7.0	0.12	0.40	0.0	0.0	0.15	0.5	0.04	0.14	0.05	0.16	0.007	0.020
11–18–24	6:00 p.m. to 8:30 a.m. In cage	120	5.8	0.80	0.96	0.04	0.04	0.04	0.05	0.03	0.04	0.13	0.16	0.060	0.080
	8:30 a.m. By catheter	50	6.2	2.07	1.03	0.20	0.10	0.04	0.02	0.04	0.02	0.07	0.03	0.060	0.030

after marked diuresis produced by organic mercury compounds. Such findings indicate a retention as well as a diminished content of these ions in the tissues.

Comparison of findings in blood and urine. The maximal excretion of glucose occurred during the diuretic period, when the concentration of blood sugar was highest. A rapid fall of the sugar in the blood with a corresponding rapid decrease of the sugar in the urine and volume of the urine were characteristic of the later diuretic period. Similar relationships between the sugar in the blood and that in the urine and the volume of urine have been accurately estimated by Felsher and Woodyatt (6). Because of the lack of a simple method for estimating saccharose in the blood and urine, these amounts were not estimated. The rapid excretion of urea during diuresis was associated with a fall in the blood content. The increased urea content of the blood at the height of water loss was accompanied by very low concentration of urea in the urine. Following the administration of water equal to that lost, there was a fall to the control level in the blood and a corresponding rise in the urea concentration in the urine. A high chlorid concentration in the plasma during water loss was associated with a low concentration in the urine. Similar findings were also observed with phosphorus and occasionally with sodium. The subsequent intake of water, equal to that lost, caused a decrease from the high serum concentration of chlorid, phosphorus and sodium, but little or no increase in the excretion of these substances in the urine, which is quite contrary to that observed with urea.

Experiments in which toxic phenomena developed. Four animals died before the completion of the experiment. In two (dogs G607 and H403) (tables 1 and 2) twitching movements and convulsive seizures occurred within from a few minutes to an hour after the injection of sugar had been completed, and continued periodically until death, from one to four and three-quarter hours later. The body temperature rose in dog G607 from 97.8° to 110.2°F. (36.5° to 43.4°C.) (figs. 3 and 6) and continued high after death, but no greater water loss occurred than in dog H714 in which the temperature rose 3.8°F. (2.1°C.). The results of the experiment on dog H403 were similar to those on dog G607, although the convulsive seizures began later; the body temperature did not rise so high, 4.5°F. (2.5°C.) and the water loss was not so great. The results in these two experiments were similar to those reported by Balcar, Sansum and Woodyatt (7). Glucose was given to dog G607 and saccharose to dog H403 in amounts no greater than in numerous previous experiments, and the sugars were from the same stock. The rise in hemoglobin was no greater than in other experiments but definite acidosis occurred in dog G607. At necropsy on this dog congestion of the mucous membrane of the upper portion of the small intestine and three tapeworms were found.

In dog G403 the only visible gross lesion was congestion of the gastric mucosa. In these two experiments the rubber tubing used during the injection was of poor grade, was not detoxicated by boiling in a solution of sodium hydroxid, nor were strict aseptic precautions used. These results were similar to those reported previously in which the sugar solutions had been sterilized by boiling and non-aseptic technic was employed; these details are mentioned in order to emphasize the importance that any possible toxic factor may play in causing the hyperpyrexia, convulsive seizures and death, in an animal with acute diuresis (2), (3).

Experiments on dogs H415 and H581 (tables 1 and 2) are of special interest, because the same careful detailed technic was employed as in the four successful experiments and yet the animals died in from five and a quarter to eight and three-quarter hours after completion of the injection. They gradually passed into a condition of progressive asthenia without twitching or convulsive movements; the body temperature did not rise more than 2.1°C. (the highest rise recorded in the four successful experiments), nor was the water loss or the increase in hemoglobin concentration of the blood greater. Acidosis was present in dog H415, but not in dog H581. Two hundred sixty-five cubic centimeters of water were administered to dog H415 without restorative effect two hours before death. At necropsy on dog H415 mild enteritis of the small intestine was found, and it was literally filled with round-worms. In dog H581 there was only a single round-worm and mild gastro-enteritis.

When death occurred during these experiments the abnormal findings were usually, but not necessarily, hyperpyrexia, convulsive movements and a tendency toward acidosis of the blood. Other metabolic changes differed little from those in the animals that recovered. Except for the presence of intestinal worms and the congestion of the mucous membrane of the stomach and small intestine no extensive gross pathologic changes were present.

It should be pointed out that in the four experiments on dogs H581, H714, G607 and H403, in which the body temperature rose above 2°C., the excretion of chlorin in the urine was distinctly increased over that of the control period. In dog G607, with the most marked hyperpyrexia, the increase over the control period amounted to 4.8 grams. In three of these experiments (dogs H581, H714, H403) there was also an associated increase in the amount of sodium excreted.

Discussion. Marked hyperpyrexia did not occur in this series of experiments, when care was taken to exclude all possible toxic factors connected with the preparation and administration of the solutions of sugar. On the other hand it did occur when such toxic factors were not eliminated. We know that the amount of sugar injected must be close to the lethal dose, and that a sudden loss of 10 per cent of the water of the body is close to

the limit of the loss possible without death resulting. Thus a minute amount of toxic material can play an important part in causing untoward symptoms and rapid death. These facts have led us to believe that the convulsive seizures and high body temperature that occurred in two experiments were due to toxic material in the solution rather than to the amount of sugar injected or the water lost from the organism. However, death occurred after the injection of solutions of sugar when it was known that toxic material was not present. In these experiments there were no convulsive seizures and no marked hyperpyrexia but gradual circulatory failure. The gross loss of water was no greater than in animals that recovered with the subsequent administration of water. The simplest explanation for these deaths would seem to be that these particular dogs were either unable to assimilate so large an amount of sugar or to survive such great water loss. If this assumption is correct water loss without marked hyperpyrexia can cause a fatal result.

A moderate increase in body temperature bore no direct relation to the sugar injected, whether saccharose or glucose. Neither was there any absolute relationship between the rise in temperature and the amount of water lost. In this connection some recent experiments of Boyd, Hines and Leese (8) with solutions of 30 per cent glucose show that rises in temperature comparable to those observed in the four dogs in the present series that recovered can occur without gross loss of water. The condition in the urine produced by an excessive intake of water, such as the decrease in the hydrogen-ion concentration and in the amount of ammonia and phosphorus, is similar in many respects to that after diuresis, as is the fall in concentration of urea and chlorid with an increase in total output. The traces of phosphorus in the urine during water loss may indicate a possible increase in its excretion from the intestine under such conditions. The output of fixed inorganic base in the urine, as evidenced by the amount of sodium and potassium excreted, was usually, but not invariably, increased. These findings were in striking contrast to the large amount of inorganic base excreted when diuresis was produced by acid-forming salts, as calcium chlorid, shown by Gamble, Blackfan and Hamilton (9), or organic mercury compounds, as novasurol, shown by Keith and Whelan (10). Marked loss of water from the body is thus not necessarily accompanied by a relatively marked loss of fixed inorganic base.

There was always evidence during water loss of a decrease in the volume of circulating blood. In spite of this factor predisposing to a shift in the acid-base equilibrium toward the acid side, acidosis in the blood was the exception. The increase of urea and chlorid in the plasma may be partly due to concentration of the blood; however, failure of normal excretion from the kidneys must also be considered as a possible factor.

Comparison, during these experiments, of some of the common con-

stituents of the blood plasma and urine was of interest. When glucose was injected, the period of maximal level in the blood corresponded to the period of maximal excretion in the urine. During the period of water loss the blood sugar gradually fell to normal, while the amount excreted in the urine was very small. Boyd, Hines and Leese (8) have shown that metabolism is decidedly increased at this period, which indicates that some of the excess of glucose in the tissues is rapidly burned, thus explaining in part the quick decrease in blood sugar. During diuresis the urea output increases with a fall in the plasma concentration. With the establishment of marked water loss the plasma content rose and the amount in the urine fell. This condition is probably due to impairment of renal excretion, which in turn is due to circulatory changes and to a decrease in the water content of the blood. The rapid restoration to normal of the urea content in plasma and urine with administration of water is most striking. Such findings indicate that the kidney was little damaged by water loss.

The concomitant changes in chlorid content in blood plasma and urine were in marked contrast to those of urea. At the height of water loss in the plasma there was an increase in the plasma chlorid and marked decrease in urine content, whereas with restoration of water equivalent to that lost, the plasma chlorid showed a decided decrease but the urine content remained extremely small. The logical explanation of these later changes would seem to be that water loss had produced retention of the remaining chlorids in the tissues and that much of the added water was likewise held in the tissues. This failure of excretion of chlorids when considerable water had been administered to the organism following acute diuresis points to retention in tissues rather than to renal impairment. This difference in the excretion of chlorid and urea subsequent to a large output of water in the urine is characteristic of many different diuretic substances.

CONCLUSIONS

1. Marked rapid water loss can occur without marked hyperpyrexia.
2. During diuresis produced by saccharose or glucose there is an increase in the total excretion of urea, chlorid and sodium.
3. The plasma urea, chlorid and sodium are increased at the end of the diuretic period in consequence of the concentration of the blood.
4. With restoration of water, the plasma, urea, chlorid and sodium return to normal and the excretion of urea in the urine continues, but there is a retention of chlorid and sodium.

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